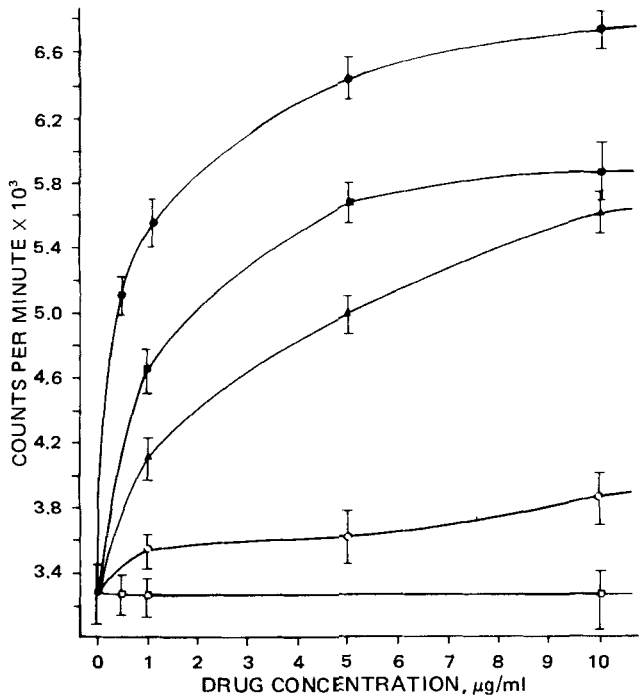

Figure 4—*Radioactive count curves for other drugs with the morphine antibody. Standard deviations are included. Key: ●, morphine; ■, dihydromorphine; ▲, heroin; ○, meperidine; and □, methadone, methadone metabolite, propoxyphene, norpropoxyphene, apomorphine, and papaverine.*

contained a very small amount of an opiate. These cases were detected by radioimmunoassay but, due to occasional variations in the extraction technique and flame-ionization detector response, the opiate gave a GLC response too small to be considered positive.

Urine samples spiked with various concentrations of methadone, methadone primary metabolite, propoxyphene, norpropoxyphene, apomorphine, dihydromorphine, hydrocodone, hydromorphine, heroin, papaverine, oxycodone, and berberine (goldenseal) also were tested. Heroin, dihydromorphine, hydromorphine, hydrocodone, oxycodone, and high concentrations of meperidine reacted with the radioimmunoassay opiate antibody (Fig. 4). All of these drugs could be differentiated from morphine, codeine, or nalorphine by the two GLC columns (Table I).

This radioimmunoassay–GLC method offers a sensitive and reliable confirmation procedure for ascertaining the presence or absence of codeine and morphine in urine on a large scale.

REFERENCES

(1) E. G. C. Clarke, "Isolation and Identification of Drugs," Pharmaceutical Press, London, England, 1969, pp. 164, 165, 269, 431, 432.

(2) J. T. Payte, J. E. Wallace, and K. Blum, *Curr. Ther. Res.*, **13**, 412 (1971).

(3) J. E. Wallace, J. D. Biggs, J. H. Meritt, H. E. Hamilton, and K. Blum, *J. Chromatogr.*, **71**, 135 (1972).

(4) J. Cochin and J. W. Daly, *Experientia*, **18**, 294 (1962).

(5) S. Spector and C. W. Parker, *Science*, **168**, 1347 (1970).

(6) "Abuscreen," Roche Diagnostics, Division of Hoffmann-La Roche, Nutley, N.J.

(7) S. J. Mulé, M. L. Bastos, and D. Jukofsky, *Clin. Chem.*, **20**, 243 (1974).

(8) S. J. Mulé, E. Whitlock, and D. Jukofsky, *ibid.*, **21**, 81 (1975).

(9) E. Schmezler, W. Yu, M. S. Hewitt, and I. J. Greenblatt, *J. Pharm. Sci.*, **55**, 155 (1966).

(10) J. E. Wallace, J. D. Briggs, and K. Blum, *Clin. Chem. Acta*, **36**, 85 (1972).

(11) F. Fish and W. D. Wilson, *J. Chromatogr.*, **40**, 164 (1969).

(12) N. C. Jain, R. D. Budd, and T. C. Sneath, in "CRC Methodology for Analytical Toxicology," I. Sunshine, Ed., CRC Press, Cleveland, Ohio, 1975, pp. 271–274.

(13) K. E. Rubenstein, R. S. Schneider, and E. F. Ullman, *Biochem. Biophys. Res. Commun.*, **47**, 846 (1972).

(14) "EMIT Opiate Assay," Syva Corp., Palo Alto, Calif., 1973.

(15) N. C. Jain, T. C. Sneath, R. D. Budd, and W. J. Leung, *Clin. Chem.*, **21**, 1486 (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 22, 1975, from the *Departments of Pharmacology and of Community Medicine and Public Health, University of Southern California Medical School, Los Angeles, CA 90033, and the ‡Rancho Los Amigos Hospital, Downey, CA 90242.

Accepted for publication March 10, 1976.

ˣ To whom inquiries should be directed (at Rancho Los Amigos Hospital).

Use of Rabbits for GI Drug Absorption Studies

TADAO MAEDA ˣ, HIROSHI TAKENAKA, YOSHIYA YAMAHIRA, and TAKESHI NOGUCHI

Abstract □ A novel procedure to control the stomach emptying rate in rabbits is presented. Rabbits were given a special solid diet for 1 week, and then the gastric contents were washed out with saline. Then the rabbits were muzzled to prevent coprophagy during the night. Fifty grams of special soft diet given to the "stomach-emptying-controlled" rabbit transferred exponentially from the stomach into the small intestine and almost disappeared from the stomach within 5 hr. Griseofulvin, indomethacin, or nalidixic acid was administered in a hard gelatin capsule or tablet, with subsequent feeding of a special soft diet. Good correlations were observed between the plasma level–time curves of these drugs in the stomach-emptying-controlled rabbits and in human subjects.

Keyphrases □ Absorption, GI—griseofulvin, indomethacin, and nalidixic acid, effect of stomach emptying rate, rabbits □ GI absorption—griseofulvin, indomethacin, and nalidixic acid, effect of stomach emptying rate, rabbits □ Stomach emptying rate—effect on GI absorption of griseofulvin, indomethacin, and nalidixic acid, rabbits □ Griseofulvin—GI absorption, effect of stomach emptying rate, rabbits □ Indomethacin—GI absorption, effect of stomach emptying rate, rabbits □ Nalidixic acid—GI absorption, effect of stomach emptying rate, rabbits

In the study of GI absorption of drugs, various *in vitro* or *in situ* methods have been widely used (1–4). From the physiological point of view, the experimental animal data obtained by these destructive or operative techniques have only limited value in predicting drug absorption characteristics in humans, although such data provide funda-

Table I—Composition of Solid Diet for Rabbits

Composition	Content, %	
	Commercial Solid Diet (Pellets)	Special Solid Diet (Pellets)
Crude fiber	17.5	3.9
Crude protein	18.6	21.4
Crude fat	3.4	3.5
Crude ash	10.4	4.5
Water content	5.1	6.0
Nitrogen-free extract	45.0	60.7

mentally useful information on drug absorption.

The principal purpose of this study was to clarify, by use of intact animals, the correlation of GI drug absorption of orally administered drugs between humans and animals so that the need for human trials in developing drugs and dosage forms of increased bioavailability can be minimized. Rabbits are often used for this purpose because of their low cost and ease in handling.

According to a relevant study (5), solid material remains in the rabbit stomach even after fasting for more than 24 hr. Moreover, the stomach emptying time of rabbits differs greatly from that of humans, whose stomachs usually empty within several hours following ingestion of food. Therefore, the rabbit is not a suitable animal for the evaluation of GI absorption characteristics of drugs, especially when stomach emptying is a limiting factor (5).

The present study was designed to examine the stomach emptying characteristics of rabbits and to establish a method for simulating the stomach emptying rate to that of humans so that rabbits could be used for studies of GI drug absorption.

EXPERIMENTAL

Materials—Two kinds of solid diet, commercial[1] and special[2], were used for the rabbits (Table I). The special solid diet was prepared by removing alfalfa from the commercial solid diet. A special soft diet was prepared by adding 60 parts of water to 40 parts of special solid diet. This mixture was left standing overnight in a cool place to swell and was then divided into required amounts and made into balls.

Both griseofulvin USP and indomethacin NF were micronized to less than 5 μm in size using a fluid energy mill[3] and capsulated in a hard gelatin capsule size No. 3. Nalidixic acid NF was milled to about 30 μm utilizing a pulverizer[4] and administered as a tablet. Each tablet contained 250 mg of nalidixic acid, 55 mg of lactose USP, 16 mg of calcium carboxymethylcellulose JP, 3.5 mg of starch USP, and 3.5 mg of magnesium stearate USP. The tablets met the USP disintegration test. The other chemicals were of reagent grade.

Procedure of Stomach-Emptying Control—White male rabbits, 2.4–3.4 kg, were fed the special solid diet in place of the commercial solid diet for 1 week of conditioning before the absorption study.

After being fasted overnight with water given freely, the rabbits were anesthetized by injection of 0.3–0.5 ml/kg of 5% pentobarbital sodium. A rubber stomach tube, 25 cm in length and 5 mm in external diameter with a large hole on the side of the tip, was inserted into the stomach, and 50–100 ml of warm saline (37°) was instilled. The fluid in the stomach was then withdrawn by suction with a syringe. This procedure was repeated until the fluid withdrawn hardly contained any solid material. The rabbits usually recovered from anesthesia within 2–3 hr.

After gastric lavage, the rabbits were allowed water *ad libitum* and were muzzled to prevent coprophagy during the night. On the following morning, the rabbits were given 200 g of the special soft diet and allowed water *ad libitum*. They were muzzled again at night until morning. These pretreated rabbits were called the "stomach-emptying-controlled" rabbits.

Table II—Effect of Diet and Muzzling on Stomach Emptying

Diet	Weight of Gastric Content[a], g	
	Coprophagy Not Restricted	Muzzled during the Night
Commercial pellet diet	97 ± 7.9	33 ± 4.5
Special soft diet	83 ± 6.5	34 ± 3.9

[a] Data represent average and *SE* for rabbits of each group.

Estimation of Coprophagy—Four groups, each consisting of three rabbits, 2.4–2.8 kg, were designated as Groups I-A, I-B, II-A, and II-B. The first two groups were fed 100 g of the commercial diet daily and allowed water *ad libitum* for 3 days. Group I-A was not restricted from coprophagy, while Group I-B was muzzled during the night. Similarly, Groups II-A and II-B were fed 250 g of the special soft diet daily and allowed water *ad libitum* for 3 days. Group II-A was allowed coprophagy, while Group II-B was muzzled during the night. On the morning of the 4th day, the rabbits were sacrificed. The stomach was removed, and the amount of gastric contents was determined by subtracting the weight of the gastric pouch from that of the total stomach.

In another experiment, the effect of muzzling on the amount of feces excreted was examined using stomach-emptying-controlled rabbits. Fifteen stomach-emptying-controlled rabbits, 2.5–3.0 kg, were divided equally into Groups A, B, and C. Each rabbit was housed separately in a cage with a double floor. The upper wire mesh floor was designed to allow feces to drop through so that they could be collected on the lower wire mesh floor. The experiment schedule employed was as follows.

Gastric lavage was performed for all rabbits at 11:00 am. Groups A and B were muzzled for 17 hr during the first period (4:00 pm–9:00 am), while Group C was not muzzled at all. Feces were weighed at the end of this first period. At the beginning of the second period of 7 hr (9:00 am–4:00 pm), every rabbit was fed with 200 g of soft special diet. Only Group A was then muzzled until 4:00 pm (for about 6 hr); Groups B and C were not muzzled. At the end of the second period (4:00 pm), feces excreted were weighed again for all rabbits. During the third period of 17 hr (4:00 pm–9:00 am), Groups A and B were muzzled but Group C was not muzzled. At the end of this period, feces were again weighed for all rabbits. Thus, the total muzzled period was 40 hr for Group A, 34 hr for Group B, and 0 hr for Group C.

Stomach Emptying of Special Soft Diet—Each of the 15 stomach-emptying-controlled rabbits, 2.5–2.7 kg, was fed 50 g of the special soft diet and was allowed water *ad libitum* until sacrifice. At 0.5, 1, 2, 3, and 5 hr after feeding, three rabbits were sacrificed.

The stomach was immediately removed, the contents of the stomach were centrifuged, and the precipitates were dried to constant weight *in vacuo* at 60°. The weight of the gastric residue was calculated on the basis of the weight of the special soft diet dried under the same condition.

Administration of Drugs—To each rabbit, an oral dose of 10 mg/kg of griseofulvin or indomethacin was administered in a capsule. An oral dose of 250 mg of nalidixic acid was administered as an intact tablet. Prior to administration of either the capsule or tablet, rabbits were secured to an animal board and the mouth was pried open with a wooden rod. Then the tongue was pulled out by rolling the wooden rod and pressed down so that the capsule or tablet could be easily placed on the radix linguae and pushed down with a rubber stomach tube to the esophagus. This administration was followed by feeding of 50 g of the special soft diet to the stomach-emptying-controlled rabbits and 20 g of commercial solid diet to the conventionally fasted rabbits. Rabbits not consuming this amount of diet within 5 min were excluded from further studies, being considered not to be in good health. During the blood sampling period, rabbits had free access to water but not to feed, and a blood specimen was drawn at the predetermined time for analysis.

In every drug administration experiment, two groups of five rabbits were used. One group was the stomach-emptying-controlled rabbits; the other group consisted of rabbits fed with commercial solid diet and then fasted but allowed water *ad libitum* for 24 hr prior to the experiment. Rabbits of the latter group are called the conventionally fasted rabbits.

Analytical Method and Equipment—Blood specimens obtained by

[1] Commercial solid diet CR-1, Nihon Clea Co., Aobadai 2-20-14, Meguro, Tokyo, Japan.
[2] Nihon Clea.
[3] GemT jet mill, Helme Chemicals Inc., Helmetta, NJ 08828.
[4] ECK atomizer, Fuji Paudal Corp., Joto-ku, Osaka, Japan.

Table III—Amount of Feces Excreted and Effect of Muzzling in Preventing Coprophagy

Group[b]	Amount of Feces Excreted[a], g: First Period (4 pm–9 am)	Second Period (9 am–4 pm)	Third Period (4 pm–9 am)	Total
A	4.3 ± 2.1	14.3 ± 3.2	14.1 ± 3.2	32.7 ± 4.1
B	4.9 ± 3.2	2.4 ± 0.6	16.8 ± 2.8	24.1 ± 2.7
C	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.8 ± 0.2

[a] Data represent average and *SE* for rabbits of each group. [b] Group A rabbits were muzzled during the first, second, and third periods; Group B rabbits were muzzled during the first and third periods; and Group C rabbits were not muzzled at all.

cardiac puncture, using a heparinized syringe, were centrifuged to obtain plasma for analysis. Griseofulvin was extracted with ether from plasma and measured quantitatively by a gas chromatograph[5] equipped with a ^{63}Ni-electron-capture detector. Diazepam was used as an internal standard, and the method of Shah *et al.* (6) was followed.

The chromatographic column was a 1.5-m × 3-mm glass tube packed with 10% SE-30 on Chromosorb W AW DMCS. The column temperature was 270°, the injection port temperature was 300°, the detector block temperature was 310°, and the carrier gas (99.999% nitrogen) flow rate was 60 ml/min. Indomethacin in plasma was determined spectrofluorometrically[6], using a modification of the assay method of Hucker *et al.* (7). In this experiment, 0.01 *N* sodium hydroxide was used in place of 0.1 *N* sodium hydroxide, thus improving the analytical reproducibility in a low concentration of indomethacin. Nalidixic acid in plasma was spectrofluorometrically determined by the method of McChesney *et al.* (8).

RESULTS AND DISCUSSION

In considering stomach emptying of experimental animals, the effect of diet properties and the habit of coprophagy cannot be ignored. For laboratory animals, a solid pellet diet is convenient and popular. The refuse obtained during soybean-curd preparation[7], containing more than 60% water, has been widely used as rabbit feed in Japan. Rabbits do well on this food and require no water.

Preliminary experiments on stomach emptying of diet in the stomach-washed-out rabbits showed that the soybean curd refuse moved into the small intestine faster and more smoothly than the commercially available solid pellet diet. This result may be reasonably explained because the digestive function of the stomach is the formation of chyme and its transfer to the duodenum.

For these reasons, after gastric lavage the rabbits were fed the special soft diet resembling the soybean-curd refuse to satisfy the purpose of this study. Chiou *et al.* (5) reported that the stomachs of rabbits after fasting for 24 hr were almost as full as those of unfasted animals, although the rabbits were maintained on a wire mesh support which allowed feces to drop to the bottom of the cage, thus reducing coprophagy.

Two kinds of feces are excreted by rabbits: round and hard pellets, often seen in the cage, and soft pellets covered by a mucous material. During the night, rabbits take the latter directly from the anus and never take feces dropped on the floor (9, 10). Therefore, it was expected that the effective prevention of coprophagy would result in rather fast and smooth stomach-emptying of the rabbit gastric contents, although the quantitative estimation of coprophagy has not been reported. In this study, a muzzle was adopted for the rabbit to prevent the mouth from making a direct contact with the anus.

The effect of muzzling on the amount of gastric contents was examined (Tables II and III). The amount of the gastric contents of the nonmuzzled rabbits (Groups I-A and II-A) was about twice that of the muzzled rabbits, indicating that a fair amount of the contents was attributable to coprophagy (Table II).

Some soft fecal pellets, which were probably swallowed intact early in the morning, were found in the gastric contents of the nonmuzzled rabbits regardless of diet. The gastric contents of the muzzled rabbits were less solid in consistency but still contained hair, which might have retarded the stomach emptying of food. The amount of residual gastric

[5] Shimadzu GC-5APTFE.
[6] Hitachi MPF-2A.
[7] Okara.

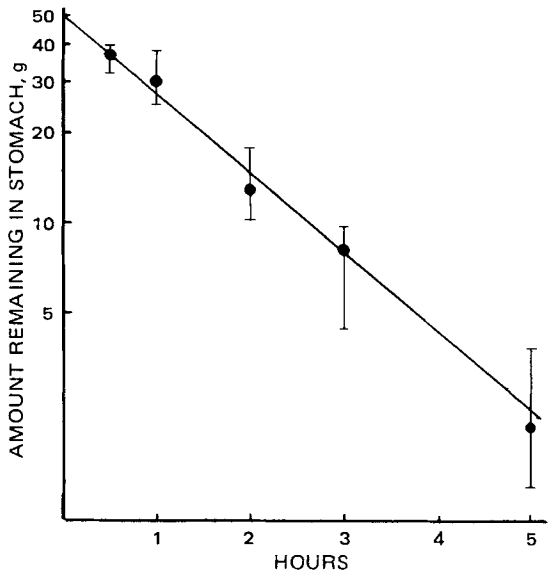

Figure 1—*Stomach emptying pattern of special soft diet (50 g) in the stomach-emptying-controlled rabbits. Each point represents the mean observation and the range on three rabbits.*

contents was not significantly affected by the type of diet. However, the gastric contents of rabbits fed the special solid diet were washed out more easily than those of rabbits fed the commercial solid diet. Therefore, the special solid diet was used during the conditioning period before performing GI absorption studies. In addition, gastric dissection confirmed that no solid material remained in the stomach when the gastric lavage procedure was employed.

The coprophagous amount and the gastric contents were examined using stomach-emptying-controlled rabbits. As shown in Table III, Group C rabbits, who were not restricted from coprophagy, hardly dropped any feces, even after ingesting 200 g of the special soft diet at the beginning of the second period. Group A rabbits, who were muzzled except during feeding time, excreted about 30 g of feces. It was reconfirmed that rabbits took feces directly from the anus as they were being excreted. Group B rabbits, who were not muzzled for several hours after feeding during the second period, ingested about 12 g of feces. This ingestion occurred during the daytime, although it had been believed to occur primarily at night. These findings demonstrate the strong physiological habit of coprophagy in rabbits, the difficulty of completely preventing coprophagy under customary conditions, and also the effectiveness of the muzzle.

After the third period, *i.e.*, after fasting for about 23 hr, all rabbits were sacrificed and the gastric contents of each group were examined. The stomachs of Group C rabbits were full of a wet mass as a result of coprophagy, although gastric lavage had been performed as in Groups A and B. For this reason, Group C was considered not suitable for GI absorption study. The stomachs of Group A rabbits were almost completely empty, and those of Group B contained some solid material, but the contents were so slurred that no difficulty would be expected in the transfer of the contents into the duodenum.

Stomach emptying in the stomach-emptying-controlled rabbits was estimated after feeding of 50 g of the special soft diet. Stomach emptying of diet appeared to be exponential in pattern (Fig. 1), and almost all ingested feed was transferred into the small intestine within 5 hr. This phenomenon is similar to the results of Hunt and Spurrell (11) with humans. The fact that such good semilogarithmic plots of amount were found with rabbits and resembled results with humans suggests that stomach emptying in these rabbits is sufficiently controlled for the GI absorption study of drugs with limited or regional solubility.

Griseofulvin is reported to be absorbed irregularly and incompletely from the GI tract because of its extremely low solubility (12). According to one study (13) on the GI absorption of this drug in rabbits, the blood level peaks occurred 6–11 hr after administration; in humans, the blood level peaks usually occurred approximately 2–3 hr after administration (14). However, Fig. 2 shows that the maximum plasma level was observed 2 hr after administration in the stomach-emptying-controlled rabbits.

Individual data of this experiment showed that the distribution of the plasma peak time observed from 1 to 2 hr after administration was characteristic in the stomach-emptying-controlled rabbits, while indi-

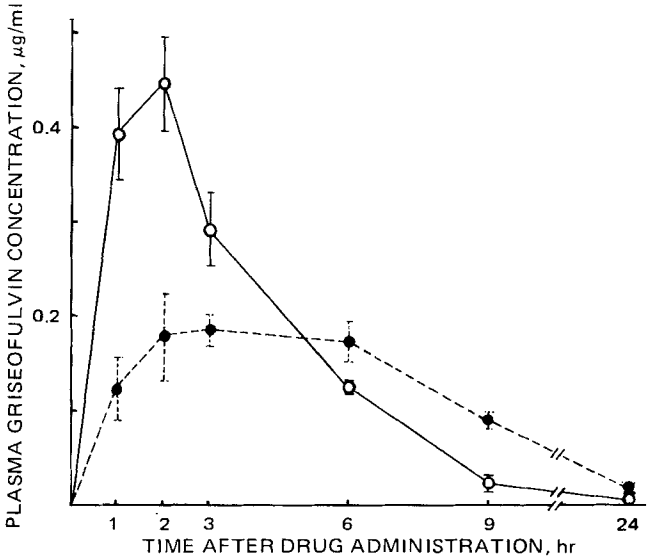

Figure 2—*Plasma levels of griseofulvin following oral administration of 10 mg/kg in the stomach-emptying-controlled rabbits (○) and rabbits fasted for 24 hr by the conventional method (●). Each point represents the mean observation ± SE on five rabbits.*

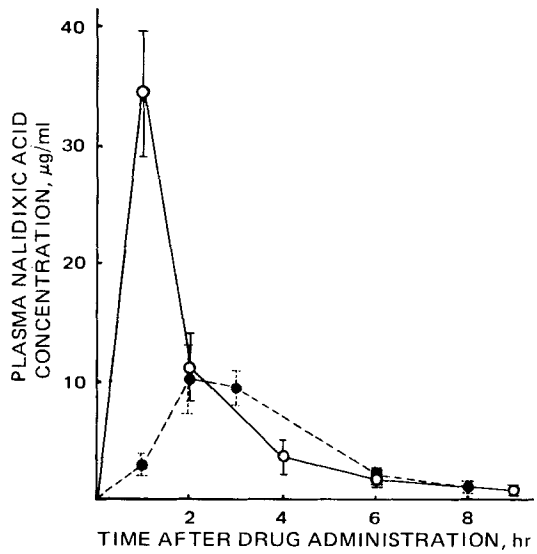

Figure 4—*Plasma levels of nalidixic acid following oral administration of 250 mg as a tablet to the stomach-emptying-controlled rabbits (○) and rabbits fasted for 24 hr by the conventional method (●). Each point represents the mean observation ± SE on five rabbits.*

vidual plasma peak time in the conventionally fasted rabbits ranged from 2 to 6 hr after administration. These results suggest that the slow and prolonged absorption of griseofulvin reported previously (13), after an oral dose of 150 mg/kg was administered as a suspension, was ascribable to an overdose and to the peculiarity of stomach emptying in rabbits, as pointed out in a later report (5).

Indomethacin absorption from the GI tract depends upon its holding time in the stomach and is retarded when stomach emptying is slow (15). The peak plasma level in the stomach-emptying-controlled rabbits prominently appeared in 1 hr, whereas slow absorption of the drug was observed in the conventionally fasted rabbits (Fig. 3). In the latter group, individual peak plasma time ranged from 2 to 5 hr after administration. The difference in the apparent absorption rates of indomethacin between the two groups of rabbits reflected the effect of the gastric emptying control procedure, because the water solubility of indomethacin is quite limited in the acidic pH range of the stomach (16). The fast absorption seen in the stomach-emptying-controlled rabbits is comparable to the

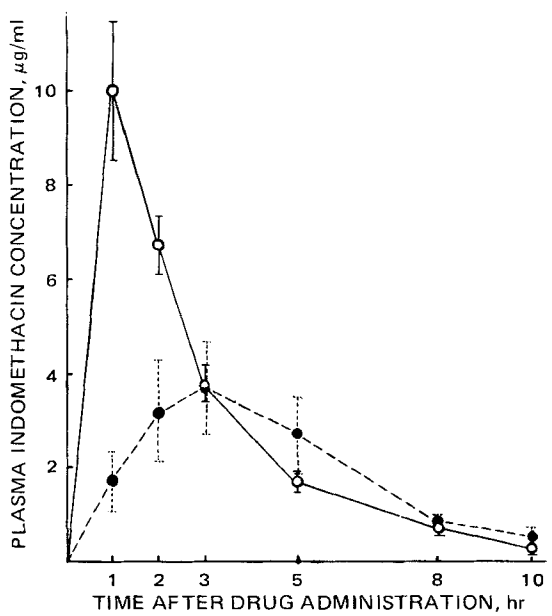

Figure 3—*Plasma levels of indomethacin following oral administration of 10 mg/kg in the stomach-emptying-controlled rabbits (○) and rabbits fasted for 24 hr by the conventional method (●). Each point represents the mean observation ± SE on five rabbits.*

results observed in humans whose peak plasma level occurred 1 hr after drug ingestion (7).

Plasma level curves in Fig. 4 demonstrate that absorption of nalidixic acid in the stomach-emptying-controlled rabbits was rapid. All individual plasma level curves showed a peak 1 hr after administration and the rapid disappearance of the drug from plasma thereafter, comparable to the results observed in humans (8). Individual peak plasma time in the conventionally fasted rabbits ranged from 2 to 6 hr.

Table IV summarizes the comparison of the individual peak plasma level, C_{max}, and its time of occurrence, t_{max}, following oral administration of griseofulvin, indomethacin, and nalidixic acid between two groups of rabbits. Fast and smooth transfer of drugs to the intestine in the stomach-emptying-controlled rabbits resulted in a statistically significant increase in the average C_{max} as compared to that in the conventionally fasted rabbits.

The t_{max} parameter also reflects the drug absorption rate. As mentioned previously, the individual t_{max} of the stomach-emptying-controlled rabbits for the three drugs was within 1–2 hr after drug administration while that of conventionally fasted rabbits ranged from 2 to 6 hr. Since the distribution range of individual t_{max} values differed between the two groups, it is quite obvious, even without statistical analysis, that the average t_{max} of the stomach-emptying-controlled rabbits was significantly less than that of the conventionally fasted rabbits.

The retardation and variation of drug absorption seen in the conventionally fasted rabbits are presumably due to the decrease and difference in the rate in which gastric contents transferred to the intestine, because

Table IV—Comparison of Peak Plasma Levels and Its Time of Occurrence following Oral Administration of Drug in Stomach-Emptying-Controlled and Conventionally Fasted Rabbits

Drug Administered (Dose)	Average of Individual Peak Plasma Level[a], C_{max}, μg/ml: Stomach-Emptying-Controlled Rabbits	Average of Individual Peak Plasma Level[a], C_{max}, μg/ml: Conventionally Fasted Rabbits	p Value[b]
Griseofulvin (10 mg/kg)	0.51 (1.6)	0.24 (3.4)	<0.01
Indomethacin (10 mg/kg)	10.47 (1.2)	5.48 (2.8)	<0.05
Nalidixic acid (250 mg/rabbit)	34.2 (1.0)	12.5 (3.2)	<0.01

[a] Numbers in parentheses are the averages of individual peak time (t_{max}, hr). [b] Determined by Student t test.

their stomachs should have been full of solid material as a result of coprophagy.

The data presented here show that the stomach-emptying-controlled rabbit can be a satisfactory model for evaluation of GI absorption characteristics of these drugs with limited or regional solubility.

CONCLUSION

Chiou *et al.* (5) concluded that the rabbit was not a useful animal for drug absorption studies because it was almost impossible to obtain an empty stomach in the rabbit by using the conventional fasting method and because the fasted state markedly prolonged the stomach emptying time. This conclusion has been very instructive to many investigators. However, in this study it was found possible to simulate the stomach emptying rate of rabbits to that of humans by taking adequate steps to empty the stomach and to feed the animals with a special soft diet. When the stomach emptying rate is controlled, the rabbit can be used as an animal model for predicting bioavailability of oral dosage for human subjects.

Results of bioavailability studies on oral dosage forms will be reported later.

REFERENCES

(1) T. H. Wilson and G. Wiseman, *J. Physiol.*, **123**, 116 (1954).

(2) L. S. Shanker, P. A. Shore, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **120**, 528 (1957).

(3) L. S. Shanker, D. J. Tocco, B. B. Brodie, and C. A. M. Hogben, *ibid.*, **123**, 81 (1958).

(4) S. Feldman, M. Salvino, and M. Gibaldi, *J. Pharm. Sci.*, **59**, 705 (1970).

(5) W. L. Chiou, S. Riegelman, and J. R. Amberg, *Chem. Pharm. Bull.*, **17**, 2170 (1969).

(6) V. P. Shah, S. Riegelman, and W. L. Epstein, *J. Pharm. Sci.*, **61**, 634 (1972).

(7) H. B. Hucker, A. G. Zacchei, S. V. Cox, D. A. Brodie, and N. H. R. Cantwell, *J. Pharmacol. Exp. Ther.*, **153**, 237 (1966).

(8) E. W. McChesney, E. J. Froelich, G. Y. Lesher, A. V. R. Crain, and D. Rosi, *Toxicol. Appl. Pharmacol.*, **6**, 292 (1964).

(9) C. E. Adams, "The UFAW Handbook on the Care and Management of Laboratory Animals," 4th ed., Churchill Livingstone, London, England, 1972, p. 167.

(10) K. W. Hagen, "The Biology of the Laboratory Rabbit," Academic, New York, N.Y., 1974, p. 33.

(11) J. N. Hunt and W. R. Spurrell, *J. Physiol.*, **113**, 157 (1951).

(12) C. Bedford, D. Busfield, K. J. Child, I. MacGregor, P. Sutherland, and E. G. Tomich, *Arch. Dermatol.*, **81**, 735 (1960).

(13) L. J. Fischer and S. Riegelman, *J. Pharm. Sci.*, **54**, 1571 (1965).

(14) M. Rowland, S. Riegelman, and W. L. Epstein, *ibid.*, **57**, 984 (1968).

(15) M. Moriyama, M. Saito, S. Awazu, M. Hanano, and H. Nogami, *Yakugaku Zasshi*, **91**, 1217 (1971).

(16) T. Fuwa, T. Iga, M. Hanano, H. Nogami, and M. Kashima, *ibid.*, **91**, 1223 (1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 18, 1975, from the *Formulation Research Department, Pharmaceuticals Division, Sumitomo Chemical Co., Ltd., Kurakakiuchi 1-3-45, Ibaraki City, Osaka 567, Japan.*

Accepted for publication March 11, 1976.

Presented at the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, Japan, April 1974.

The authors thank Prof. H. Sezaki of Kyoto University for guidance and advice and Mr. Zenzo Harada for technical assistance.

[x] To whom inquiries should be directed.

Kinetics of Hydrolysis of Fenclorac

C. M. WON [x], J. J. ZALIPSKY, D. M. PATEL, and N. H. REAVEY-CANTWELL

Abstract □ The kinetics of hydrolysis of fenclorac were studied to determine its stability in aqueous solution at different pH's and temperatures. For this study, a stability-specific liquid chromatographic assay method was developed to separate fenclorac from its hydrolysis product, α-hydroxy-3-chloro-4-cyclohexylbenzeneacetic acid. The *k*–pH profile in the 0–12 pH range in various buffer solutions shows that fenclorac is stable in its undissociated form in strongly acidic media and is unstable in neutral and alkaline media. The instability of fenclorac in aqueous solution is proportional to the degree of ionization of the carboxyl group in the 1–4 pH range and is independent of pH above 4. The rate-determining step in the mechanism of hydrolysis of fenclorac involves ionization of the carbon–chlorine bond. The ionization is catalyzed by an intramolecular nucleophilic attack on the α-carbon by the dissociated carboxyl group, resulting in the formation of an unstable intermediate, a three-membered ring lactone. This unstable intermediate rapidly hydrolyzes to the final hydrolysis product. This mechanism is supported by experimental evidence such as the medium effect, positive salt effect, common ion effect, and substituent effect. Arrhenius parameters for the hydrolysis of fenclorac and its 3-nitro substituted analog were obtained.

Keyphrases □ Fenclorac—kinetics of hydrolysis, stability in aqueous solution, various pH's and temperatures, liquid chromatographic analysis □ Hydrolysis—fenclorac in aqueous solutions, kinetics, various pH's and temperatures, liquid chromatographic analysis □ Stability—fenclorac in aqueous solutions, various pH's and temperatures, liquid chromatographic analysis □ Liquid chromatography—analysis, fenclorac in aqueous solutions □ Anti-inflammatory agents—fenclorac, kinetics of hydrolysis, stability in aqueous solution, various pH's and temperatures, liquid chromatographic analysis

In the search for a new, nonsteroidal, anti-inflammatory compound for clinical utility, a series of substituted benzeneacetic acids was synthesized (1). Fenclorac (I) (α,3-dichloro-4-cyclohexylbenzeneacetic acid) diethylammonium salt exhibited promising anti-inflammatory activities. The anti-inflammatory activity of fenclorac was demonstrated in rats using the carrageenan paw edema assay[1].

A simple, sensitive analytical method was needed to follow the hydrolysis of relatively unstable fenclorac in

[1] G. Nuss *et al.*, to be published.