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Research Article

Investigation of the Gastrointestinal Transit and *In Vivo* Drug Release of Isosorbide-5-Nitrate Pellets

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An oral formulation of controlled-release isosorbide-5-nitrate pellets has been used to investigate the location of pellets in the gastrointestinal (GI) tract and, in parallel, to measure the drug absorption from these locations. Using the method of gamma scintigraphy the transit times and spreading of pellets in the GI tract have been determined. The method of numeric deconvolution was applied to calculate the drug input into the systemic circulation. The results indicate that a well-absorbed substance such as isosorbide-5-nitrate is absorbed from the stomach and small intestinal in a manner that is controlled by the properties of the pellets. Drug absorption is reduced in the colon. The average transit time from mouth to colon is 6 to 8 hr, which represents the maximum acceptable time for drug release for this oral controlled-release preparation. Taking into account these relations an isosorbide-5-nitrate pellet formulation with a bioavailability of 84% has been developed that maintained the minimal therapeutic plasma level for more than 16 hr after application.

KEY WORDS: controlled-release pellets formulation; gamma scintigraphy; gastrointestinal transit time; isosorbide-5-nitrate; absorption rate; numerical deconvolution.

INTRODUCTION

One aim in developing a controlled-release drug formulation is to reduce the frequency of drug administration. This can be achieved by maintaining the therapeutic plasma concentration for an extended period of time. The once-daily administration of a drug formulation resulting in a constant plasma level is often thought to be optimal. However, problems can occur for the individual case where tolerance effects preclude a constant plasma level over a period of 24 hr. Furthermore, a circadian rhythm of the plasma levels is required with some active substances (organic nitrates, corticosteroids), which should be achieved by the release profile of the drug.

A drug that requires defined plasma-level fluctuation in order to avoid tolerance phenomena is isosorbide-5-nitrate (IS-5-N). On the other hand, a controlled-release preparation for a once-daily application is useful, and therefore a prolonged drug release must combine an optimal time of drug release that assures a good bioavailability with a release profile that provides a tolerance-avoiding plasma-level profile. This study defines such a time course of drug release from IS-5-N formulations without a significant loss of bioavailability.

Sustained-release IS-5-N shows varying bioavailabilities according to the extent of retardation of release. For example, in a study conducted by Simbec (1), a depot formulation providing sustained release *in vitro* over 14 hr showed a relative bioavailability of approximately 60%. If the *in vitro* period of retardation is reduced to 8 hr, the bioavailability rises to 84% (2). IS-5-N is reported to be well absorbed (up to 100%) from the upper gastrointestinal tract and is not affected by first-pass metabolism (3). Therefore, reduction in bioavailability of a controlled-release form could result from a decrease in absorption or metabolism in the lower intestinal regions (colon).

In order to investigate how long the release of IS-5-N can be retarded without a loss of bioavailability (i.e., the area of gastrointestinal tract in which IS-5-N is absorbed), the gastrointestinal transit and *in vivo* dissolution/absorption of IS-5-N from Elantan long pellets (Pharma Schwarz, GMBH) were studied. One capsule of Elantan long contains 50 mg of IS-5-N as a sustained-release formulation. The distribution and residence time of radiolabeled Elanton long pellets in the gastrointestinal tract were determined by gamma scintigraphy. The analysis of plasma samples drawn at the same times as the scintigraphic images allowed calculation of the time-dependent input of the IS-5-N in correlation with the locations of the pellets.

MATERIALS AND METHODS

Manufacture of ^{111}In-Labeled Controlled-Release Pellets

Sugar beads (0.6–0.71 mm in diameter) were coated with a solution of ^{111}In-DTPA complex in a specially designed small-scale Wurster apparatus. One milliliter of

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^{111}InCl solution (0.05 *M* HCl; radioactive concentration, 15.0 mCi/ml; radioactive purity, 99.98%; New England Nuclear, D-6072 Dreiech) was mixed with 5 μl of DTPA solution (100 μg DTPA; Merck, D-6100 Darmstadt). This solution was diluted with 6 ml ethanol, containing 3.125 mg ethylcellulose, and was sealed onto the beads with a water-impermeable lacquer consisting of ethylcellulose, polyvinylacetate, and talc (leakage rate, 1.1% at 1 hr and 25.5% at 4 hr; water, 37°C; paddle apparatus). Onto this layer was added 70% of the drug substance IS-5-N, the release controlling membrane, and the loading dose of 30% IS-5-N using the Wurster equipment.

Methods

The assay of IS-5-N was by a reversed-phase high-performance liquid chromatography method (eluant, 30/70 methanol/water; column, Zorbax C18 (DuPont); flow rate, 1.5 ml/min; temperature, 40°C; UV detection, 220 nm; RT, ≈3 min).

Two dissolution methods were used: the USP XXI paddle method and the NF XIV rotating bottle method, modified.

The dissolution media were (i) artificial gastric juice, pH 1.2; (ii) phosphate buffer, pH 5.5; (iii) phosphate buffer, pH 7.5; (iv) demineralized water; (v) a solution of 0.05% polysorbate 80 in water (Atlas Chemie, D-4300 Essen; surface tension, 40 mNm^{-1}); and (vi) an aqueous solution of 2% methylcellulose (Dow Chemicals, D-6000 Frankfurt; viscosity, 42 mPa · sec). The substances were used as supplied by the manufactures, without further purification.

Description of Volunteers

The study was conducted in healthy male volunteers of different age, weight, and height; the mean age was 25.5 ± 7.4 years (range, 18–41 years). The mean weight was 69.3 ± 9.3 kg (range, 55–88 kg) and the mean height was 177.1 ± 6.9 cm (range, 165–184.5 cm). The dietary intake was controlled before and during the investigation. The study was approved by the Ethical Committee of Nottingham University and was conducted in accordance with the Helsinki Guidelines for Ethics in Research.

Administration of the Formulation

The composition of the pellets was qualitatively and quantitatively in accordance with the commercial product Elantan-long with the exception of the radioactive label. One capsule, containing radioactive labeled pellets (2 MBq/dose) and 50 mg IS-5-N, was administered, together with 100 ml water, to six male, healthy subjects after a standard breakfast comprising 1.5 bread rolls, a piece of salami sausage, 1 slice of cheese, 1 egg, butter, marmalade, and 2 cups of coffee (caloric value, 2300 kJ).

In the second part of the study (after a washout period of 1 week), each of the subjects received an Elantan 20 tablet (Pharma Schwarz GMBH, D-4019 Monheim).

Gamma Scintigraphy

In the first part of the study the subjects stood in front of a gamma camera equipped with a parallel collimator with a 40-cm field of view. Anterior and posterior views of 60-sec duration were taken at suitable times. A piece of adhesive tape containing ^{111}InCl was used as an external marker, fixed above the liver. The radioactivity was measured in the stomach and the colon regions (=regions of interest; ROI) and was corrected for the decay of radioactivity and background counts. The geometric mean of the anterior and posterior views was calculated as discussed previously (4). Plasma samples were drawn simultaneously.

Assay of Plasma Samples

Plasma samples were taken either by repeated venipuncture or with an intravenous cannula. At least 5 ml of blood was taken at the following times after the dose: 0 (predose), 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hr. Each blood sample was centrifuged and the supernatant plasma was pipetted off into glass test tubes. Plasma samples were stored at −20°C until assayed. Details of the gas chromatographic assay have been described elsewhere (5). The reproducibility was checked on five identical plasma samples, analyzed as described above, each spiked with known amounts of IS-5-N. According to these results the precision and accuracy of the method were judged as good (see Table II).

In Vivo Dissolution/Absorption of IS-5-N

The *in vivo* release profile for each subject was determined with the use of the point-area numerical deconvolution method (6).

The kinetic parameters, volume of distribution, and half-life of IS-5-N for each subject were determined from the second part of the study using Elantan 20. These parameters, after computer fitting of the data using the Nonlin program (7), were used as basal data for the deconvolution method.

RESULTS

In Vitro Dissolution of IS-5-N from Elantan long Pellets

Scanning electron micrographs of the controlling membrane of the small batch and those of a large-scale production batch prepared using a coating pan showed excellent uniformity. The profiles of the *in vitro* drug release for the

Table I. Influence of Testing Conditions on the *in Vitro* Drug Release from IS-5-N Pellets[a]

Conditions of drug release		
Model	Medium	K_0 (%/hr) ± SE
Rotating bottle	pH 1.2	8.11 ± 0.06
	pH 5.5	8.6 ± 0.08
	Water	
	σ = 72 mN/m	9.6 ± 0.10
	η = 42 mPa · sec	9.67 ± 0.04
USP XXI paddle apparatus	Water	
	13 rpm	9.78 ± 0.04
	90 rpm	9.68 ± 0.05

[a] Constant of zero-order release; σ, surface tension; η, viscosity.

Table II. Precision and Accuracy of the IS-5-N Plasma Assay by Gas Chromatography

Amount of IS-5-N added (ng/ml)	Mean amount found	No. of determinations	Coefficient of variation (%)
50	51.4	5	8.6
100	96.0	5	4.0
400	395.0	5	2.9

small-scale and commercial products were not significantly different as measured by the USP XXI paddle method (Fig. 1).

It had been shown previously with the commercial product that the drug release was nearly independent of the pH, viscosity surface tension, and hydrodynamics of the dissolution media. The radiolabeled product was evaluated similarly. The release of the retarded IS-5-N dose can be described as a zero-order process up to 80% dissolution. It can be concluded from Table I that neither pH, viscosity, surface tension variation, nor change in the dissolution model affected the drug release by more than 10% (Fig. 2). The drug release was diffusion controlled and nearly independent of environmental changes. From this it could be concluded that the *in vivo* release profile should be similar to that obtained *in vitro* if the dosage form was rate controlling *in vivo*.

Gamma Scintigraphy

Representative scintiscans showing the gastrointestinal transit of labeled pellets are shown in Fig. 3. The pellets emptied in a uniform manner from the stomach and spread within the small intestine. Evidence of regrouping at the ileocecal junction was observed with some subjects. The pellets were seen to spread well within the large intestine. The mean transit profile is given in Fig. 4. The time for the emptying from the stomach, transit in the small intestine,

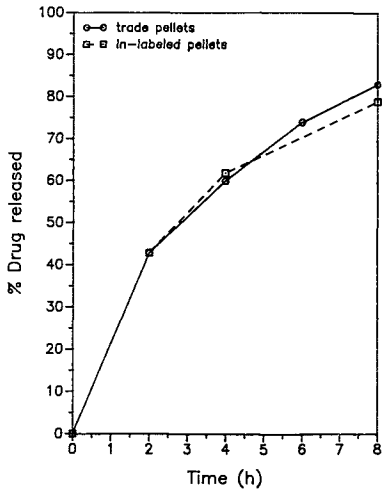

Fig. 1. Comparative *in vitro* drug release profile of ^{111}In-labeled pellets and Elantan long pellets (USP paddle method; 90 rpm; water, 37°C).

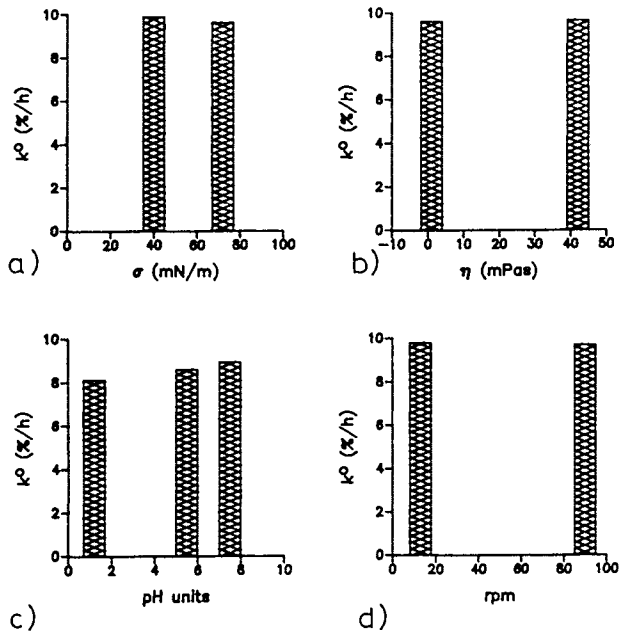

Fig. 2. Comparison of the *in vitro* drug release of IS-5-N depending on different dissolution models. $k°$, zero-order release constant. (a) Rotating bottle, influence of surface tension. (b) Rotating bottle, influence of viscosity. (c) Rotating bottle, influence of pH variation. (d) Paddle apparatus, influence of hydrodynamics.

and arrival of the pellets in the colon are given for each subject in Table III.

Emptying of the pellets from the stomach and their appearance in the colon are expressed by the time at which 50% ($T_{50\%}$) of the radioactivity is measurable in the ROI. The time for small intestine transit is calculated from the difference in these times as discussed previously (4).

The data for the gastric emptying of pellets obtained in the present study can be discussed in relation to literature data for the emptying of pellets from a fasted stomach (8,9) or following a light breakfast taken 1 hr before application. Here it was observed that pellets left a fed stomach more slowly and steadily than an empty stomach and that the gradient of the emptying curve gave an indication of the spreading of the pellets (6). After intake of the pellets on an empty stomach typical emptying times of $T_{50\%}$ from 50 to 80 min were found. Furthermore, the pellets could leave the fasted stomach as a bolus, with little or no spreading in the small intestine (8). In contrast, pellets administered to a fed stomach had gastric emptying times of 119 and 285 min, depending on the size of the breakfast administered (light versus heavy) (4,10). The mean value of 188 min found in the present work is between these two values.

The small intestine transit time of 222 min in the present study corresponds well with the mean value of 204–225 minutes reported by Davis *et al.* (4,11,12). The leakage of the label from the pellets at longer time periods may contribute to an overestimation of the spreading of the pellets system in the terminal ileum and colon. However, such leakage should not have a pronounced effect on the calculated small intestine transit time and colon arrival time since Davis *et al.* (12) have shown that the intestinal transits of pellets and solutions are very similar.

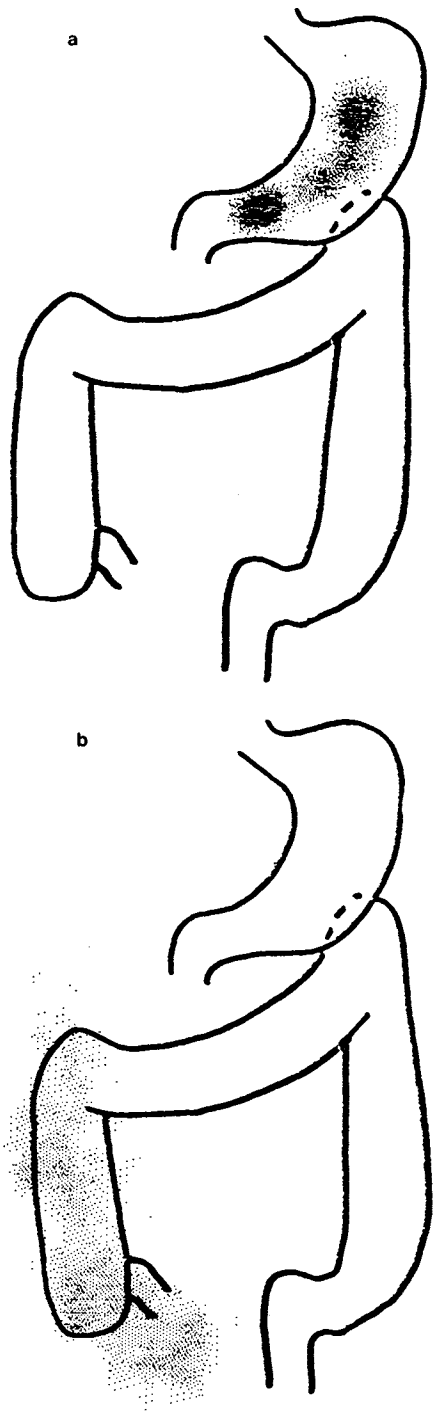

Fig. 3. Gamma scintigraphs of isosorbide-5-nitrate pellets after application following a standard breakfast. (a) Fifteen minutes after intake; the hard gelatin capsule is broken up and the pellets are dispersed in the stomach. (b) Nine hours after intake; some of the pellets are dispersed in the ascending colon, and the rest are concentrated before the valva ileocaecalis.

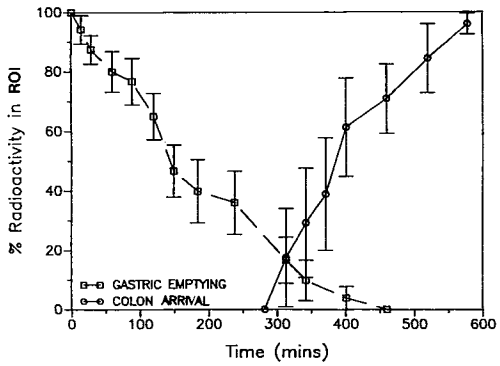

Fig. 4. Gastrointestinal transit of Elantan long pellets following a standard breakfast (mean + SE). ROI, region of interest. Left curve, stomach; right curve, colon.

Absorption Profile

Plasma-level versus time profiles are given in Fig. 5, and the *in vivo* dissolution characteristics calculated therefrom are given in Table IV. All subjects showed a rapid rise in IS-5-N level in the plasma. After 0.5 hr effective levels of over 100 ng/ml were achieved which were maintained for periods of 16–20 hr.

The results for C_{max} are in the range of 362 to 595 ng/mg, and those for T_{max} between 4 and 6 hr. The accumulated *in vivo* release/absorption amounts to 75–95% (average, 84.6%; coefficient of variation, 11.1%), which is also the relative bioavailability of Elantan long in comparison to Elantan 20 tablets (Fig. 6). The mean residence time (Table V) in comparison to Elantan 20 tablets is 2.5 hr longer.

The differential *in vivo* IS-5-N dissolution/absorption profile indicates a three-step mechanism. During the first hour approximately 25% of the applied dose was absorbed. This corresponds to the loading dose. After this initial phase a nearly constant absorption for up to 6 hr was observed. During this time approximately 40% of the dose was absorbed. In the final period, which lasts for another 12 hr, 19% of the dose was absorbed. The combined absorption of 84% of the applied dose is equivalent to the bioavailability of IS-5-N for this preparation.

It is interesting to examine the correlation between the gastrointestinal transit of the pellets and the drug input function (Fig. 7). From these data it is clear that the loading dose

Table III. Gastrointestinal Transit Times ($T_{50\%}$) (Minutes) of ^{111}In-Labeled IS-5-N Pellets

Subject No.	Emptying of stomach	Small intestine transit	Arrival in the colon
1	195	235	430
2	205	260	465
3	120	270	390
4	90	210	300
5	210	125	335
6	305	230	535
$\bar{X}$	188	222	409
SE	31	21	35

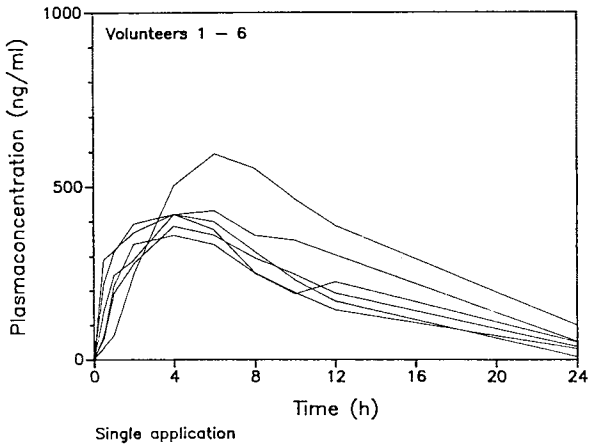

Fig. 5. IS-5-N plasma-level profiles of the volunteers after a single application of 50 mg IS-5-N as controlled-release pellets following a standard breakfast.

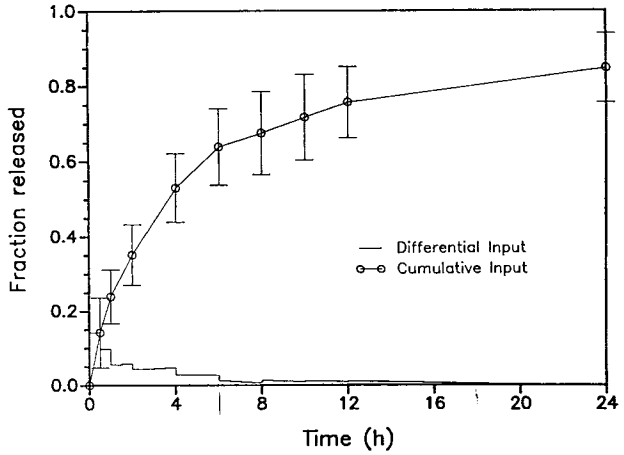

Fig. 6. *In vivo* IS-5-N release/absorption (mean + SD) as calculated by numerical deconvolution from the plasma-level profiles.

and a fraction of the controlled-release dose are released in the stomach as expected. The reduction of drug absorption that occurs 6 hr after administration can be explained by the fact that at this time about 40% of the pellets are located in the colon. The remaining 60% are spread within the stomach and small intestine. However, it is believed that the majority of these pellets is in the lower parts of the small intestine near the ileocecal sphincter. Consequently we find a reduced absorption capacity and the measured absorption rate falls. During the subsequent 12 hr the pellets spread in the colon and a continuous process of absorption takes place.

DISCUSSION

The transit of dosage forms in the small intestine is known to be reasonably constant as discussed above; therefore, the variable factor in the passage of pellets from mouth to colon is the time required for gastric emptying. This time can be specifically influenced by the intake of food. In the present study the combined residence time of the pellets in the stomach and small intestine region was 6 to 7 hr and the pellets were well spread in the jejunum and the ileum. The data obtained for the pellets in the colon region indicate that the pellets can accumulate at the junction between the small intestine and the ascending colon, i.e., at the ileocecal sphincter, and then enter the colon as one or a few boluses. Spreading of the pellets in the different regions has been reported previously.

The results of the present investigation show that there is an optimum period of time of 6 to 7 hr after intake for absorption of a well-absorbed substance such as IS-5-N. This optimal time corresponds to the residence time of the pellets in the stomach and the small intestine, where the active substance is absorbed (rate limited) according to the release profile of the pellets.

As the IS-5-N release from the controlled-release pellets is nearly independent of environmental changes, it can be concluded that the difference between *in vitro* release and *in vivo* absorption reflects the reduced absorption of IS-5-N in the lower parts of the small intestine and regions of the colon. The absorption decreases when the pellets accumulate at the ileocecal sphincter and then pass into the colon. In the subsequent 12-hr period in the colon, where distribution of the pellets occurs, only 10–20% of the applied dose was absorbed.

The results of this study show that a release time of 8 hr for IS-5-N from controlled-release preparations is the maximum time to assure nearly complete absorption of the drug. With the administration of 50 mg IS-5-N once a day, the minimal therapeutic plasma level of 100 ng/ml can be maintained for a period of approximately 18 hr. After this time the plasma level falls below 100 ng/ml, thereby avoiding tolerance phenomena (13).

Table IV. Correlation of *in Vitro* Drug Release with *in Vivo* Drug Release/Absorption of IS-5-N from ^{111}In-Labeled Pellets[a]

Time (hr)	*In vitro*	Accumulated release/absorption of IS-5-N (%) *in vivo* in subject						Coefficient of variation (%)
		RC	RW	SA	NR	WM	$\bar{X}$	
2	42.8	29	46	27	32	41	35	3.6
4	61.8	41	64	46	56	58	43	4.2
6		50	77	58	68	67	64	4.6
8	78.8	53	82	63	74	66	67.6	4.9
24	100	77	90	85	97	74	84.6	4.2

[a] Subject SW was not considered because a value for the distribution volume was not available.

Table V. Mean Residence Times (MRT) of Is-5-N After Application of Elantan long Pellets and Tablets

Subject No.	MRT (hr)	
	Elantan long	Elantan 20
1	10.59	7.39
2	9.54	6.87
3	9.99	7.25
4	8.24	6.81
5	8.29	—
6	10.92	—
$\bar{X}$ ± SE	9.56 ± 1.14	7.08 ± 0.28

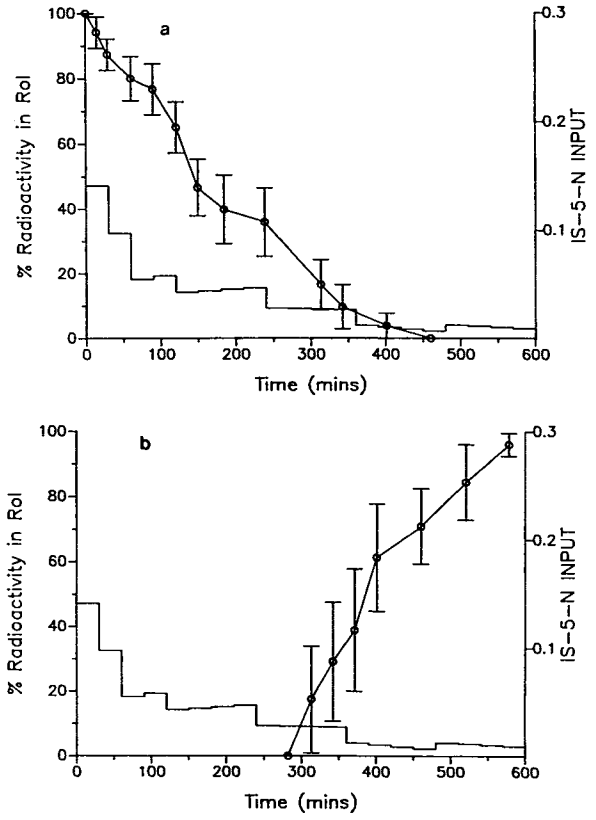

Fig. 7. (a) Differential drug input function and gastric emptying of Elantan long pellets (mean + SE). The input fraction corresponds to the right ordinate. ROI, region of interest. Upper curve, gastric emptying. (b) Differential drug input function and colon arrival of Elantan long pellets (mean + SE). Right curve, colon arrival.

For the future design of controlled-release (CR) dosage forms it can be concluded from this study that even a well-absorbed drug such as IS-5-N is poorly absorbed when it enters the colon. A CR formulation that releases the drug over the whole length of the gastrointestinal tract, i.e., stomach to colon, is sensible only if the active substance is absorbed from the colon at a rate comparable to that in the small intestine. If this requirement is not met, a loss of bioavailability can be expected. As a rule this reduced absorption will lead to a decreased plasma level, so that a twice-daily application of the controlled-release form will be required in order to provide a constant plasma level.

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