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Gastrointestinal transit and absorption of theophylline from a multiparticulate controlled release formulation

K.H. Yuen [a], A.A. Deshmukh [a], J.M. Newton [a], M. Short [b] and R. Melchor [c]

[a] *The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX (UK),* [b] *Department of Medical Physics, University College Hospital, London WC1E 6AU (UK) and* [c] *Department of Respiratory Medicine, University College Hospital, London WC1E 6AU (UK)*

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Summary

The gastrointestinal transit and absorption of theophylline from a novel multiparticulate controlled release formulation were investigated under fed and fasted conditions. The drug pellets of high drug loading were prepared using an extrusion spheronisation technique, and coated with a methylcellulose-ethylcellulose mixture to control the drug release. Transit of the dosage form in the gastrointestinal tract, determined using a gamma scintigraphic method, revealed that the presence of food delayed the gastric emptying, but was without influence on the small intestinal transit time. The delay in gastric emptying was associated with a delay in drug absorption. However, the overall rate and extent of drug absorption were essentially unaffected by the presence of food. For both fed and fasted conditions, the rate of absorption whilst the pellets were in the stomach was slower than when the pellets were in the small intestine. The pellets were less well dispersed in the stomach than in the small intestine or colon. Moreover, whereas only 14% of drug was released in the stomach, 47% was released in the small intestine. It is interesting that the remaining 39% of the drug was taken up from the colon, which thus acts as a significant site of absorption.

Introduction

The human gastrointestinal tract is a very complex organ, which can be divided into three distinct sections, namely the stomach, the small intestine and the colon. Each has its own physiological function and is very varied in terms of pH, nature of its luminal contents, length and surface area. All these variables may singly or in combination influence the drug release/absorption from a sustained release preparation.

The small intestine, with its enormous absorptive area of between 200 and 500 m^2 (Davenport, 1977), is invariably the principal site of drug absorption. In contrast, the stomach, being a secretory rather than an absorptive organ, and the colon, because of its small absorptive area, usually play a small role in the absorption of drugs. Nevertheless, particularly in the case of sustained

Correspondence to: J.M. Newton, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, U.K.

release preparations, the colon may play an important absorptive role. Although some drugs, for example, theophylline (Staib et al., 1986) and metoprolol (Godbillon et al., 1985) have been shown to be well absorbed in the colon, in general, absorption from this part of the intestine is incomplete and erratic (Koch-Weser and Schechter, 1981), since transit times through the colon are highly variable (Metcalf et al., 1987) ranging from less than 1 h to more than 60 h (Hardy et al., 1985, 1987). Absorption from the distal part can be considered negligible since any remaining drug will be embedded in semi-solid faecal matter (Hirtz, 1984).

In view of the differences in the local environment and absorptive capacity of the three sections of the gastrointestinal tract, the duration of residence and transit times of a sustained release product in each section, can greatly affect its performance. Even if a product can be formulated to release its medicament independently of the local environment, its performance can still be influenced by the orocaecal transit time if the drug is not absorbed in the colon.

The transit time through the small intestine typically takes 3–5 h, is fairly constant, and is unaffected by food status (Cammack et al., 1982; Davis et al., 1984, 1986, 1987; Devereux, 1987; Ollerenshaw et al., 1987; Mundy et al., 1989). On the other hand gastric emptying is very variable, and is influenced by diet (Christian et al., 1980; Moore et al., 1981; Davis et al., 1984, 1987; Devereux, 1987), drugs (Kaus and Fell, 1984; Lake-Bakaar and Teblick, 1984), posture (Fell et al., 1982; Hunter et al., 1982; Bennet et al., 1984) and also exercise (Cammack et al., 1982). It follows that the orocaecal transit time is highly dependent on gastric emptying. Hence, by prolonging the gastric residence, the overall transit time of a dosage form can be extended. If the drug dissolves in the stomach contents, drug solution will then pass in an unimpeded manner to the small intestine for subsequent absorption at the optimal site.

Certain sustained release theophylline products were shown to exhibit incomplete and erratic absorption (Boroda et al., 1973; Weinberger et al., 1978; Hurwitz et al., 1987). Whilst such erratic performance might be an inherent fault of the formulation itself, food, particularly that with high fat content, has been reported to affect the absorption of some of these preparations. A number of studies have shown that the effects of food are very variable. Both the rate and extent of absorption of the commercial preparation 'Theo-Dur Sprinkle' (Key Pharmaceuticals Inc., U.S.A.) were considerably reduced when taken with food (Pedersen and Moller-Petersen, 1984; Karim et al., 1985). Similar decreases in bioavailability were also reported with 'Theolong' (Eisai Co. Ltd, Tokyo) and 'Theodur-G' (Mitsubishi Chemical Industry Co. Ltd, Tokyo): the effect was more pronounced with fat-rich diets (Tada et al., 1989). Some products, however, only exhibited a delayed or decreased rate of absorption, while the amount absorbed remained unchanged when administered with food. This has been shown with 'Theo-Dur' – Key Pharmaceuticals Inc., U.S.A. (Leeds et al., 1982; Osman et al., 1983; Spector, 1985, 1986; Spector et al., 1985), 'Slo-Bid Gyrocaps' – Rorer Pharmaceuticals, U.S.A. (Hendeles and Weinberger, 1986), 'Theolair SR' – Riker Laboratories Inc., U.S.A. (Pedersen, 1981; Pedersen and Moller-Petersen, 1982) and 'Somophyllin CRT' – Fisons Corp., U.S.A. (Pedersen and Moller-Petersen, 1985). Finally, with 'Theograd' (Abbott Laborataries, U.K.), although food appeared to decrease the rate of absorption, the total amount absorbed was increased (Lagas and Jonkman, 1983, 1985).

Assessment of controlled release formulations of theophylline would therefore benefit from simultaneous studies of position of the drug in the gastrointestinal tract, and determination of content in the serum, and forms the basis of this study.

Materials and Methods

Pellet preparation

Two types of pellets were required for the experiments: (1) a controlled release theophylline preparation, and (2) an equivalent pellet formulation to be used as a carrier for gamma-emitting

radionuclide, to act as a marker for gastrointestinal transit. Both types of pellets were made by extrusion spheronisation. The former consisted of a mixture of anhydrous theophylline BP (BASF, U.K., Ltd), microcrystalline cellulose (Avicel PH101, FMC Corp.) and lactose (BP Fine grade, Dairy Crest) to give a final theophylline concentration of 80% w/w. The latter consisted of Amberlite CG400 ion-exchange resin 100–200 wet mesh (Aldrich Chemical Co. Ltd, U.K.), microcrystalline cellulose (Avicel PH101) and lactose, to give an ion-exchange resin content of 5%. Both preparations were manufactured by firstly mixing the ingredients with a suitable amount of water, extrusion of the wet powder mass through a 1 mm die using a ram extruder (Harrison et al., 1985) followed by spheronisation of the extrudates on a 208 mm plate spheroniser (Caleva, Ascot, Berks) with a radially grooved plate rotating at 1000 rpm. The wet spheroids were collected and dried in a fluid bed drier at 60°C for 1 h. The dried spheres were separated into size fractions by sieving and the most frequently occurring fraction, 1.18–1.4 mm, selected for further use.

Pellet coating

The two types of pellets required slightly different coating formulae. The theophylline pellets required a coat which provided a diffusion control of drug release. On the other hand, the Amberlite containing pellets required a coat which would allow uptake of the radiolabel into the pellet, but remain intact throughout gastrointestinal transit. The basis of the coat in both cases was ethylcellulose used in the form of Ethocel AQ (Dow Chemicals, U.S.A.). The theophylline pellets were coated with a mixture of Ethocel AQ and methylcellulose of viscosity grade 400 cp (BDH Chemicals Ltd) in an aeromatic AG Strea 1 fluidised bed coater (ACM Machinery, Tadley). The amount of methylcellulose used was 16% of the weight of the ethylcellulose. A coat which was 4.1% of the weight of the pellets was found to give a suitable in vitro release profile (Fig. 1). The drug release which was insensitive to pH changes between 1 and 7 was also stable after storage for 1 year.

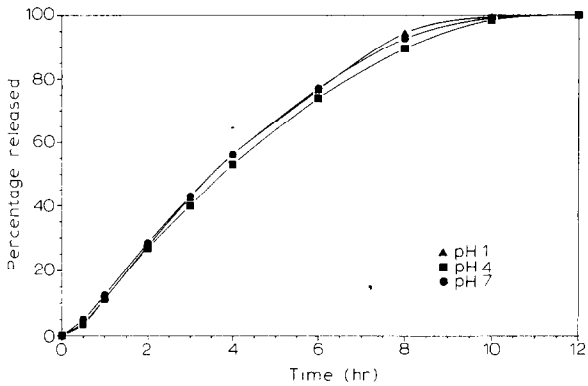

Fig. 1. In vitro theophylline release of the preparation as a function of pH ($n = 6$).

The Amberlite containing pellets were coated with a mixture of Ethocel AQ and methylcellulose of viscosity grade 15 cp (Fluka Chimie AG, Switzerland) to give a final coat which was 5.8% of the pellet weight. The amount of the methylcellulose used in this case was 40% of the weight of the ethylcellulose.

In vitro theophylline release studies

The in vitro theophylline release of the pellets was determined using the paddle unit (method 2) of the USP XXI dissolution test apparatus (model PTWS, Pharma Test Apparatebau, Germany). All the tests were conducted in 900 ml of dissolution medium maintained at 37.0 ± 0.5°C with a paddle rotation speed of 100 rpm. In each case, the weight of theophylline pellets used was 300 mg. Samples of 3 ml volume were withdrawn at various predetermined time intervals using an automated sampler (Pharma Test Apparatebau Type PTFC1, Germany). The drug concentration of the samples was determined by direct measurement of the UV absorbance at 273 nm using a Perkin-Elmer 554 UV-Vis. spectrophotometer after appropriate dilution. Preliminary experiments have established a linear relationship between drug concentrations and absorbance values. Each test was run in sets of six and the average percentage released over time was then calculated. Different dissolution media were used and they included 0.1 N hydrochloric acid, and phosphate buffer BP of pH 4 and pH 7.

Radiolabelling of Amberlite containing pellets with ^{99m}Tc

The radionuclide ^{99m}Tc was eluted daily from a commercial generator (Elumatic III, CIS, France). A sterile pyrogen free solution of sodium pertechnetate ($Na^{99m}TcO_4$) in normal saline was obtained from the elution. The film coated Amberlite containing pellets (400 mg) were soaked in 3 ml of the ^{99m}Tc solution containing approx. 200 MBq of activity. After 0.5 h of soaking, the solution was removed and the pellets washed twice with 5 ml of normal saline solution. They were then soaked for another 1 h in 10 ml of normal saline whilst rotating on a vertical gantry at 10 rpm in a shielded water bath (Townson and Mercer Ltd, Surrey) at 37°C. This was to ensure that the unbound radiolabel was rinsed off from the surface of the pellets. The solution was removed and the pellets dried, overnight, in an oven at 50°C. The following morning, the pellets were weighed and assayed for ^{99m}Tc activity. All assays of the ^{99m}Tc activity (above 1 MBq) were performed using an isotope assay ionisation chamber (Pitman Instrument, Surrey, Model 270).

In vitro testing of ^{99m}Tc binding to Amberlite containing pellets

Six batches of pellets weighing 400 mg each were radiolabelled as described above. After labelling, each batch was placed in a separate glass tube containing 20 ml of simulated gastric juice (USP XX 1980, omitting pepsin) of pH 1.2. The tubes were sealed and placed on a vertical gantry rotating at 10 rpm in a shielded water bath at 37°C. After 4 h, the pellets were removed and the remaining gastric juice in each glass tube was assayed for ^{99m}Tc activity. The radioactivity released into the supernatant was calculated as a percentage of the initial activity after correcting for decay. The experiment was repeated with phosphate buffer BP of pH 4 and simulated intestinal buffer (USP XX 1980, omitting pancreatiri) of pH 7.5. However, in these two experiments, the radioactivity released into the supernatant was determined after the pellets had been soaked in the fluids for 14 h rather than 4 h because it was felt that this would approximate the in vivo transit time of the pellets to reach the lower part of the gastrointestinal tract(colon). The pellets were found to be intact at the end of all three experiments and the percentage of activity released was found to be 2.1 ± 1.1, 2.5 ± 1.5 and $3.7 \pm 1.5\%$, respectively.

The pellets were further examined for distribution of radioactivity within each capsule. Two batches of pellets weighing 400 mg each were radiolabelled as described above. After drying for 4 h at 50°C in an oven, each batch was divided into 10 portions. Each portion was weighed and its radioactive level determined. The percentage deviation of radioactivity of each sample from the mean radioactivity of all the samples was calculated after correction for weight variations and found to average 2.8 ± 2.0 and $3.8 \pm 2.2\%$.

Study design

This was approved by the ethical committee of University College Hospital Medical School. 12 non-smoking male volunteers, between the ages of 19 and 24 years and weighing from 56 to 83 kg, participated in the study after providing written informed consent. All declared themselves healthy, were not taking any medication and had no history of gastrointestinal disorders. The volunteers were randomly divided into two groups of six volunteers each and administered the preparation after overnight fast or after a light breakfast. The dose was equivalent to 300 mg theophylline and was administered together with an equivalent weight (395 mg) of the Amberlite containing pellets, labelled with 7.4 MBq of activity, in a size OOO hard gelatin capsule (Elanco qualicaps, Lilly Industries, U.K.). The preparation was taken with 150 ml of water.

Food intake

The first group of volunteers was dosed after an overnight fast. For the second group, the preparation was taken immediately after a standard breakfast comprising 23 g cornflakes with 230 ml semi-skimmed milk and two pieces of white bread spread with 15 g butter and 20 g marmalade. The breakfast is similar to the meal used by Devereux (1987) and has a caloric value of 535 kcal. At 200 min after dosing, all volunteers were given a standard lunch of a MacDonalds' Quar-

terpounder hamburger, french fries (medium), an apple and a cup of orange juice. Supper, consisting of chicken with rice, was provided at 560 min after dosing. Ingestion of alcohol and xanthine-containing food or beverages was prohibited for 24 h before, during and 36 h after commencement of the study.

Blood sampling

Blood samples of 5 ml volume were collected in plain vacutainers at 0 (before dosing), $\frac{1}{2}$, 1, 2, 3, 4, 6, 8, 10, 14, 18, 24, 30 and 36 h after dosing. An in-dwelling cannula was used for withdrawal of blood during the first 24 h. Subsequent samples were taken by direct venupuncture. The blood samples were allowed to stand for 2 h before centrifuging for 10 min at $2000 \times g$. The serum was then transferred to separate glass containers and kept frozen until analysis.

Gamma scintigraphy

Imaging of the pellets in the gastrointestinal tract was performed using a Siemens Rota gamma camera system with two opposed ZLC 37 tube detectors, each having a 40 cm field of view and capable of simultaneous data acquisition. Both detectors were fitted with a low energy parallel hole collimator suitable for ^{99m}Tc imaging. An on-line ADAC digital computer (DPS 3300) was connected to the camera for data processing. The geometric mean response of the camera system has been assessed and validated by Devereux (1987) using a point source of ^{99m}Tc, and found to be satisfactory.

During the study, the volunteer was seated comfortably between the two heads of the camera in an upright position, leaning against the posterior detector. When the preparation was swallowed, images of 60 s per frame were immediately acquired continuously and simultaneously from the anterior and posterior detectors for 90 min. Thereafter, images were acquired at 10–15 min interval. If necessary, more frequent acquisition was made to maximise the information collected over the critical period of gastric emptying. During this time, the volunteer was allowed to move away from the camera in between imaging. The volunteer had a small sealed source of 0.6 MBq ^{99m}Tc firmly taped to the skin at the position of his lower costal margin for repositioning when the images were taken. The source was also used as an anatomical reference marker. After 6 h of imaging, the acquisition time of each image was increased to 120 s per frame, to compensate for the radioactive decay of the ^{99m}Tc. The imaging was continued until 12 h after dosing and the data collected were stored on hard disk for processing and on magnetic tape for archiving. All volunteers were also asked to report any bowel movements during the course of the study.

Analysis of serum samples

Serum levels of theophylline were measured using a reversed phase high-performance liquid chromatographic (HPLC) method. The HPLC system consisted of a Gilson model 802 manometric module, a Waters Lambda-Max Model 481 variable wavelength detector and a Gilson model 302 solvent delivery system, equipped with a Philips Pye Unicam PU 4810 integrator. The column used was a 125 mm $\times$ 4 mm stainless steel cylinder packed with 5 μm particle size Lichrosorb RP-18 (Merck) and fitted with a direct connect refillable guard column. The mobile phase comprised 1.5% tetrahydrofuran (THF) in 0.01 M sodium acetate buffer adjusted to pH 4.3 with glacial acetic acid. Analysis was run at a flow rate of 1.3 ml/min with the detector operating at 273 nm.

Prior to analysis, the drug was extracted from the serum using the following procedure. A 250 μl aliquot of serum sample was accurately measured into an Eppendorf microcentrifuge tube, followed by the addition of 50 μl of 2.5 mg/100 ml β-hydroxyethyltheophylline (BHET) internal standard solution and 1 ml of 8:2 chloroform-isopropyl alcohol extracting solvent. The mixture was vortexed for 1 min and then centrifuged at $9800 \times g$ for 1.5 min. A 0.8 ml volume of the supernatant was then removed and dried under a gentle stream of nitrogen at 60°C in a reaction vial. The residue was reconstituted with 100 μl of mobile phase and a 20 μl volume was injected onto the column. All the samples were analyzed in duplicate and the average value calculated.

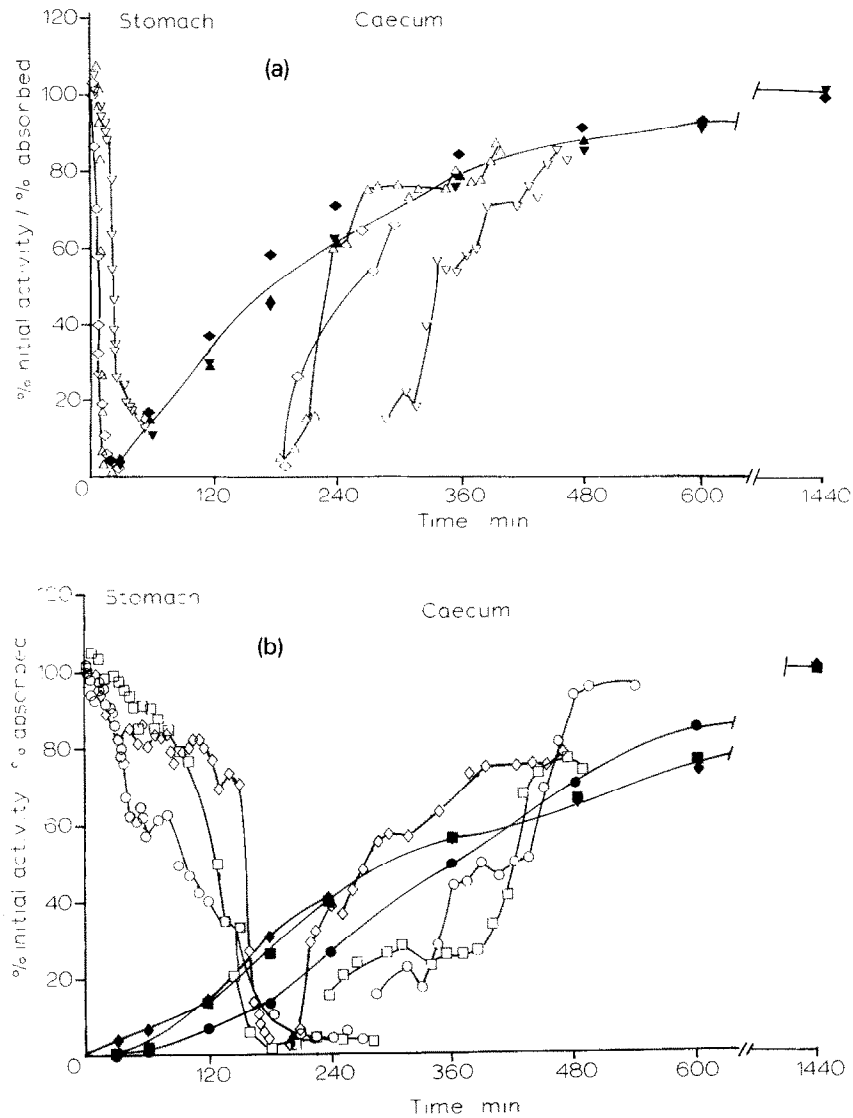

Fig. 2. Gastric emptying and caecum arrival of pellets and absorption profiles for theophylline pellets administered to volunteers when fasted.

	Volunteer	% initial activity (allowing for decay)	% theophylline absorbed
(a)	SL	▽	▼
	NA	△	▲
	KI	◇	◆
(b)	JE	○	●
	JA	□	■
	VK	◇	◆

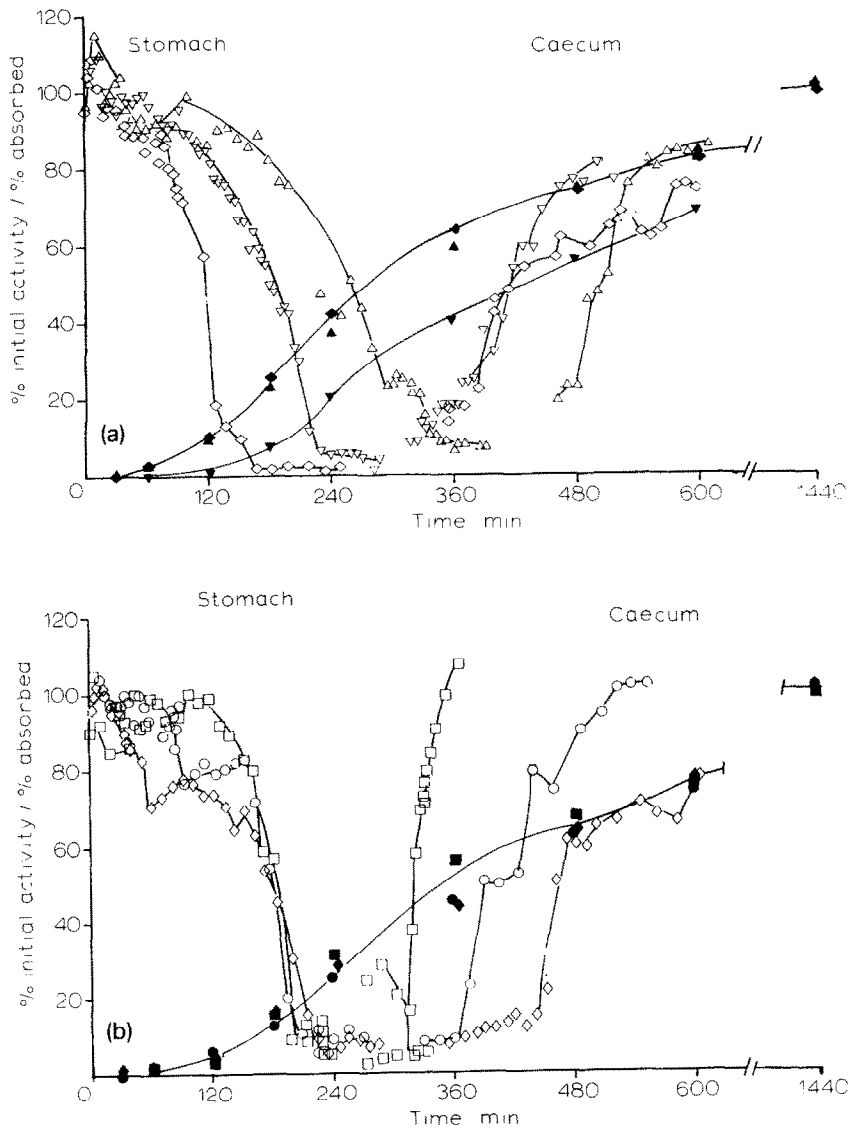

Fig. 3. Gastric emptying and caecum arrival of pellets and absorption profiles for theophylline pellets administered to volunteers when fed with light breakfast.

	Volunteer	% initial activity (allowing for decay)	% theophylline absorbed
(a)	WW	△	▲
	KN	▽	▼
	AM	◇	◆
(b)	KY	○	●
	LM	□	■
	WI	◇	◆

Theophylline standards were prepared by spiking drug-free serum in a concentration range of 1–8 μg/ml. Standard curves, recoveries and precision studies were performed using these serum standards. The recovery values for both theophylline and the internal standard (BHET) were greater than 93%, whilst the coefficients of variation were smaller than 4% for both within-day and between-day assays in this concentration range. In addition, detector linearity, determined with theophylline standards prepared in water in a concentration range of 0.5–16 μg/ml, was found to be linear. The related xanthines, theobromine, para-xanthine and caffeine, when injected onto the column under the same analytical conditions were well separated from the peaks of interest.

Analysis of scintigraphic data

The images acquired for each volunteer were played back on the computer. Three areas of interest were drawn on the computer screen with a light pen, representing the stomach, large intestine and anatomical marker. This was done for both the anterior and posterior images. The full sequence of images was viewed to check for movement of the volunteer by referring to the anatomical marker. If, on some frames, the subject had moved excessively, these frames were ignored during subsequent analysis. The counts recorded for each area of interest by each camera head were calculated by the computer for each image. These values were corrected for different acquisition time periods and background counts. The background correction was made by subtracting, from each pixel in the area of interest, the mean counts per pixel from a region at the edge of each image. To correct for variation in depth and attenuation of the radioactivity, the geometric mean count was calculated from these net counts and corrected for decay (Tothill et al., 1978). Finally, the corrected geometric mean counts for the stomach and caecum regions were expressed as percentages of the total counts recorded initially when all the administered activity was in the stomach region. The time course of gastric emptying and caecum arrival of pellets in each volunteer could then be estimated from the plot of percentage activity in these two regions vs time.

Analysis of serum data

The serum concentration data were analyzed using the Wagner-Nelson method (1964) to estimate the percentage absorbed versus time profiles of the preparation administered under fed and fasted conditions. The method is based on a one-compartment pharmacokinetic model and is appropriate for theophylline, since its kinetic analysis can be satisfactorily applied using this model (Loughnan et al., 1976; Mungall, 1983). The k_e values (elimination rate constant) required in the calculation using the Wagner-Nelson equation were estimated from the terminal slope of individual serum level curves. Results of a previous in vivo study indicated that the in vivo release/absorption of the formulation was complete by 24 h after dosing (Yuen, 1991). Therefore, if it can be assumed that the absorption of the preparation was complete by 24 h, the k_e values could be reliably estimated from the three terminal concentration values (at 24, 30 and 36 h after dosing) by logarithmic transformation of the data and application of linear regression (Gibaldi and Perrier, 1982). Prior to the calculation, the linearity of the terminal slope was inspected by plotting the values on semilog graph paper. If more than three points appeared to be linear, all these would be used for estimation of the k_e.

Results and Discussion

Gastric emptying

The curves depicting the gastric emptying and caecum arrival of pellets for the individual volunteers are shown in Figs 2 and 3. Wide intersubject variations were observed with respect to both the gastric emptying and caecum arrival curves. However, on closer examination of the figures, it is revealed that a majority of the volunteers dosed in the fed state exhibited a delayed gastric emptying and caecum arrival, with an apparent shift of the curves to the right.

The gastric emptying of a single unit non-disintegrating dosage form is a simple all-or-none pro-

cess, whilst the emptying of multiunit formulations is more complex and variable. Hunter et al. (1983) observed at least five different patterns of emptying of encapsulated formulations. Therefore, it is not possible to describe the emptying process in a concise manner by fitting the data points to a single mathematical model (Clarke, 1989). In the case of liquid or solid meals, emptying usually occurs in a constant pattern. For example, liquid emptying is generally accepted as being a monoexponential process (Heading et al., 1971; Fisher et al., 1982; Harris et al., 1987). On the other hand, it has been suggested that digestible solids empty in a linear fashion with time (Heading et al., 1971; Harris et al., 1987) subsequent to a lag phase, during which the solid particles are reduced to a size small enough to be emptied. In view of the constancy of emptying patterns exhibited by liquid and solid meals, mathematical fitting of the data is more applicable and has been applied by Cook et al. (1975), Elashoft et al. (1982, 1983) and Kim et al. (1981).

Frequently, the gastric emptying (or caecum arrival) curves of more than one subject have been presented as a single average curve (Davis et al., 1984, 1987; Christensen et al., 1985; Ollerenshaw et al., 1987; O'Reilly et al., 1987; Urbain et al., 1989; Sugito et al., 1990), and linear and exponential patterns of emptying have been reported based on the average data (O'Reilly et al., 1987). However, such a composite curve may not be typical in shape of the individual curves, and may mask highly important variations and patterns of emptying (Devereux, 1987; Clarke, 1989). Therefore, a display of the individual curves will permit a better qualitative comparison between individuals and between dosage forms. A quantitative comparison can be made by focusing on some important features that can characterise the emptying pattern, and the estimated numerical values can then be analyzed statistically (Elashoff, 1981; Elashoff et al., 1982).

In the present study, a number of parameters were estimated from the individual gastric emptying profiles which permit a quantitative comparison between the emptying pattern under fed and fasted conditions to be made. The first parameter was the time for 50% of the pellets (activity) to be emptied from the stomach ($t_{50\%}$ emptied), interpolated from the emptying curve. Another parameter was the time to completion of gastric emptying ($t_{complete}$), and was taken to be the time when 10% of the activity remained in the stomach region. A third parameter, the gastric emptying lag time, was obtained by reference to the computer images rather than the emptying curves. It was defined as the time corresponding to that image when all detectable activity appeared to remain within the stomach region of interest. Attempts to estimate this parameter from the emptying curve were hindered by oscillations in radioactivity, probably due to redistribution of the labelled pellets within the stomach (Clarke, 1989). From the difference between $t_{complete}$ and

TABLE 1

Numerical values (min) of gastric emptying parameters

Volunteer		Lag time		$t_{complete}$		Emptying period		$t_{50\%}$ emptied	
Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed
KI	LM	7	117	16	196	9	79	11	183
JE	KN	27	145	184	315	157	170	88	179
SL	EY	20	105	41	255	21	150	24	183
NA	AM	4	59	18	165	14	106	8	118
VK	WI	120	50	215	230	95	180	154	176
JA	WW	95	160	187	380	92	220	136	246
Mean		46	106	110	257	65	151	70	181
SE		(20)	(18)	(38)	(32)	(24)	(21)	(27)	(17)
Independent *t*-test		$P = 0.049$		$P = 0.015$		$P = 0.023$		$P = 0.005$	

the gastric emptying lag time, an emptying period was estimated as the fourth parameter.

The numerical values of the parameters for individual volunteers are given in Table 1. Wide intersubject variations were observed in the numerical values of the parameters for both food statuses. In general, the values in the fed state were larger than the fasted values. For each parameter, the difference between fed and fasted values was analyzed statistically using an independent (unmatched) t-test, with the assumption that the values were normally distributed and the sample variances homogenous (Healy et al., 1986). All variances were evaluated for homogeneity using the F_{max} test (Hartley, 1950), and were all found to conform with the assumption. The results of the statistical analyses are also shown in Table 1. A statistically significant difference was observed between the fed and fasted values for all the four parameters ($P < 0.05$).

The results of the statistical analyses indicate that the gastric emptying pattern was significantly different under the fed and fasted states (Table 1). The average values of all four parameters were significantly increased when the volunteers were dosed in a fed state. Therefore, food not only delayed the onset but also decreased the rate of gastric emptying, resulting in a prolonged emptying period. Devereux (1987) reported a similar increase in the fed values of $t_{50\%}$ emptied and $t_{complete}$, with pellets of same size range and density, but no statistical difference was observed between the fed and fasted values of lag time and emptying period. Studies by Christian et al. (1980) and Davis et al. (1984, 1987) also showed that, following a heavy meal, the pellets were emptied more slowly than in a lightly fed state. On the other hand, the timing of the pellets' administration relative to eating, was reported to have no influence on the emptying rate. Pellets dosed before, during and after a meal were shown to have similar half-lives of emptying (O'Reilly et al., 1987). They further reported that pellets dosed predispersed with food, emptied at the same rate as those taken in a capsule, but dispersion within the stomach was more rapid.

Whilst it has been asserted by Beckett (1981) and Eskilson (1985) that multiunit dosage forms have the advantage of wide distribution in the stomach, the scintigraphic images obtained for both food statuses in the present study, revealed that the distribution was only limited. This is in contrast to the findings of Hardy et al. (1985) and O'Reilly et al. (1987), and may indeed be attributed to differences in the surface characteristics of the pellets studied. The anionic exchange resin beads employed by these workers were uncoated, whilst the Amberlite containing pellets used in the present study were coated with ethylcellulose. Thus, their ability to disperse could have been reduced by the more hydrophobic nature of the pellet surfaces (Devereux, 1987; Clarke, 1989). It was further observed that, in general, the pellets were emptied as a series of small boluses, an observation shared by Devereux (1987) and Clarke (1989). Nevertheless, in a number of volunteers dosed fasted, emptying occurred as a few large boluses and was complete within a short period.

The majority of gastric emptying studies (involving multiunit dosage forms), make no reference to a lag time of emptying (Clarke, 1989). The most common parameter used to describe the gastric emptying process was $t_{50\%}$. In reviewing the literature, Devereux (1987) observed vast differences in the $t_{50\%}$ values reported between the workers, and the author ascribed the discrepancies to different physical properties of the pellets studied, such as size range and density. Some batches of pellets investigated by Devereux (1987) and Clarke (1989) were similar to those studied here. For these pellets, they reported a mean fasted $t_{50\%}$ value of 125 min (range 64–211) and 185 min (range 87–477), respectively, compared to 70 min (range 8–154) obtained in the present study. Whilst the influence of food was not evaluated by Clarke (1989), Devereux (1987) reported a mean fed $t_{50\%}$ value of 181 min (range 106–218), similar to that obtained here (range 118–246). Notwithstanding the fact that the fed values were comparable, it appeared, even when the pellets were similar in size and density, that the gastric emptying rate (as defined by the $t_{50\%}$ value) could vary considerably between studies. In addition, within each study itself, the results were subject to large variations between individuals, as

demonstrated by the wide range of values obtained.

Clarke (1989) noted that some volunteers were consistently rapid emptiers, whilst some were consistently slow emptiers. Variations within subjects have also been reported by Plankey et al. (1988), and may be related to their emotional status, such as stress and anxiety, which affect the gastric emptying process (Kaus and Fell, 1984). A variety of other factors may contribute to the variations obtained within and between studies, even when the dosage forms are essentially similar in their physical characteristics. In the fasted state, the stomach generally shows minimal motor activity. However, a short period of intense contractions known as the migrating motor complex (housekeeper effect), occurs every 2–3 h and clears the stomach of fasting contents into the small intestine. Therefore, emptying of a dosage form dosed in the fasted state, is dependent on the time of administration and the time of the next housekeeper waves which occur at regular intervals in a given subject (Cortot, 1984). Since the interval between two housekeeper contractions is highly variable from one individual to another, this will further contribute to differences between subjects.

The physiological conditions in which the pellets are exposed in the stomach under the fed mode are very different from those of the fasted state, and may explain the differences observed in gastric emptying between the two food statuses. The fed state is characterised by continuous contractile activity of the antrum, which mixes and grinds the stomach contents. The rate of emptying in the fed mode varies between individuals (Heading et al., 1971) and is also influenced by the volume, osmolality and nutrient composition of the meal (Cortot, 1984). All these factors may thus cause variations in results between studies. The similarity of the mean fed $t_{50\%}$ value obtained in the present study and that of Devereux (1987) is noteworthy. This is in view of the fact that the meal used in both studies was the same, and this could have contributed to the similarity of results obtained.

TABLE 2

Numerical values (min) of caecum arrival and small intestinal transit time

Volunteer		$t_{50\%}$ arrival		Small intestinal transit time	
Fasted	Fed	Fasted	Fed	Fasted	Fed
KI	LM	239	318	228	135
JE	KN	390	413	302	234
SL	EY	342	388	318	205
NA	AM	244	412	236	294
VK	WI	279	454	125	278
JA	WW	425	503	289	257
Mean		320	415	250	234
SE		(32)	(25)	(29)	(24)
Independent *t*-test		$P = 0.042$		$P = 0.681$ (NS)	

Small intestinal transit and caecum arrival

Arrival of pellets at the caecum was described by a $t_{50\%}$ value for caecum arrival, interpolated from the caecum arrival curves. This represented the time, from ingestion, until 50% of the pellets had entered the caecum. On the other hand, the small intestinal transit time (SITT), was obtained from the difference between the $t_{50\%}$ values for caecum arrival and gastric emptying. In all the volunteers, imaging was performed over sufficient length of time to allow for more than 50% arrival of activity at the caecum. The individual results of $t_{50\%}$ arrival and SITT are given in Table 2 The difference between the fed and fasted values of each parameter was also analyzed statistically using the independent *t*-test, and the results shown in the same table.

Unlike gastric emptying, the small intestinal transit times were not affected by food, consistent with the findings of Davis et al. (1984, 1986, 1987), Devereux (1987) and Mundy et al. (1989). No statistically significant difference ($P = 0.681$) was observed between the mean fed and fasted values (of 234 and 250 min, respectively). The food induced delay in gastric emptying, was associated with a corresponding delay in caecum arrival, resulting in a fairly consistent interval between the two processes for both the fed and fasted states. Therefore, the transit of the pellets in the small intestine was independent of the gastric emptying process (Lagerlof et al., 1974;

Read, 1984; Devereux, 1987; Davis, 1989). Whilst limited dispersion was observed in the stomach, the pellets appeared to be better dispersed during transit along the small intestine, but the degree of dispersion was influenced by the gastric emptying rate. Thus, when the pellets were emptied rapidly, less spreading was observed. By contrast, better dispersion was obtained when the pellets were emptied slowly and regularly in small doses as suggested by Davis (1989).

The small intestinal transit times observed in these studies were comparable to those reported by Devereux (1987) and Clarke (1989) for pellets of similar size range and density, and those of other workers even though the pellets studied were not necessarily of the same size or density (see Devereux, 1987). Therefore, in contrast to gastric emptying, the small intestinal transit times do not show wide variations between protocols and methods. It is interesting to note that Davis et al. (1984) further reported that the small bowel transit was also unaffected by the size and shape of the dosage form.

A statistically significant difference was observed between the fed and fasted values of $t_{50\%}$ arrival at the caecum ($P < 0.05$). The mean $t_{50\%}$ value was increased from 320 min in the fasted state to 415 min in the fed state (Table 2). Similar effects of food were reported by Davis et al. (1984, 1987). Following a heavy breakfast, both the gastric emptying and caecum arrival times of the pellets were significantly increased compared to a lightly fed state. On the other hand, although Devereux (1987) observed that the caecum arrival times were increased by an average of 61 min, there was no statistical difference when compared to the fasted values.

The caecum arrival process was often characterised by accumulation of the pellets at the ileocaecal region, followed by emptying in boluses which was quite rapid in certain cases, resulting in a sharp increase in activity in the caecum (Figs 2 and 3). This pattern of caecum transfer has also been previously noted by Devereux (1987) and Spiller et al. (1986). Once in the caecum, a retrograde passage of the contents was prevented by the ileocaecal valve, and the pellets exhibited only very slow movements. Although the rates of spreading and transit in the colon were reported to be highly variable (Metcalf et al., 1987), the pellets have the potential for wide distribution (Hardy and Perkins, 1985). Wide distribution was usually observed from the images taken 24 h after dosing, but occasionally this was also observed towards the end of the imaging studies (12 h after dosing).

Gastrointestinal transit and absorption

The results presented in the foregoing sections clearly indicated that food caused a significant delay in the gastric emptying of the pellets. Indeed, this may explain the differences observed in the absorption characteristics of the formulation under the fed and fasted conditions. The mean serum level and absorption profiles of the formulation are depicted in Figs 4 and 5, together with the distribution of the pellets in the gastrointestinal tract. The demarcated areas under the curves denote the time the pellets spent in the stomach, small intestine and colon. The two boundaries represent the respective $t_{50\%}$ values for gastric emptying and caecum arrival of the pellets. It is interesting to note the association between a delay in the gastric emptying (and caecum arrival) of the pellets and a concomitant shift of the serum level and absorption curves to the right.

Compared to the stomach region the absorption rate appeared to be relatively faster when the pellets had emptied into the small intestine (Fig. 5). This is also demonstrated in the individ-

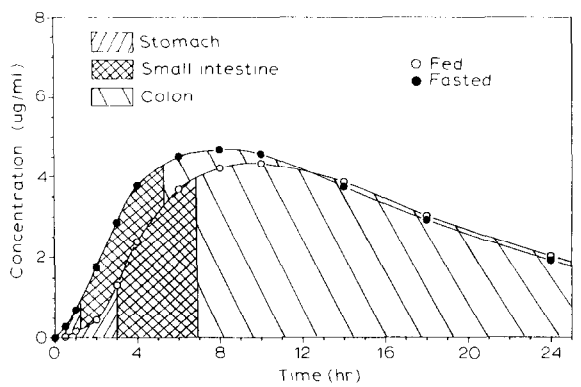

Fig. 4. Average serum theophylline concentration profiles of formula A dosed, fed and fasted, and distribution of pellets in the gastrointestinal tract.

ual absorption profiles (calculated using the Wagner-Nelson method) depicted in Figs 2 and 3, together with the gastric emptying and caecum arrival curves. Absorption was generally slow when a large proportion of pellets remained in the stomach. In contrast, a noticeable increase in absorption rate was observed when the pellets began to empty into the small intestinal region. A direct comparison of the rates of absorption in the fed and fasted conditions, indicates that food did not appreciably alter the absorption rate while the pellets were in the stomach, but merely prolonged the slow absorption phase, consequent to the delayed gastric emptying. However, because the gastric residence time was relatively brief in the fasted mode, this slow absorption phase would tend to be obscured under this condition (Fig. 2a and b). Therefore, a delay in gastric emptying would reasonably explain the existence of a slow absorption phase in the fed mode, which resulted in the serum level profile being shifted to the right. An absorption lag time was also observed in a number of volunteers, and could be attributed to a delay in disintegration of the capsule.

A number of studies on sustained release theophylline products have reported a similar consequence of food effects on the rate of absorption. Some of the products evaluated were single-unit tablets while others, such as 'Somophyilin-CRT' (Pedersen and Moller-Petusen, 1985) and 'Slo-Bid Gyrocaps' (Hendeles and Weinberger, 1986) were multiunit preparations in the form of coated granules. Although the gastrointestinal transit properties of these products were not evaluated in the studies, the observed food effects could similarly be related to the delayed gastric emptying.

Upon entering the small intestine, the absorption of the pellets appeared to occur at comparable rates between the fed and fasted conditions, as demonstrated by the parallel nature of the two absorption curves in the small intestinal region (Fig. 5). Hence, the presence of food did not influence the rate of absorption when the pellets were in the small intestine, nor was there any

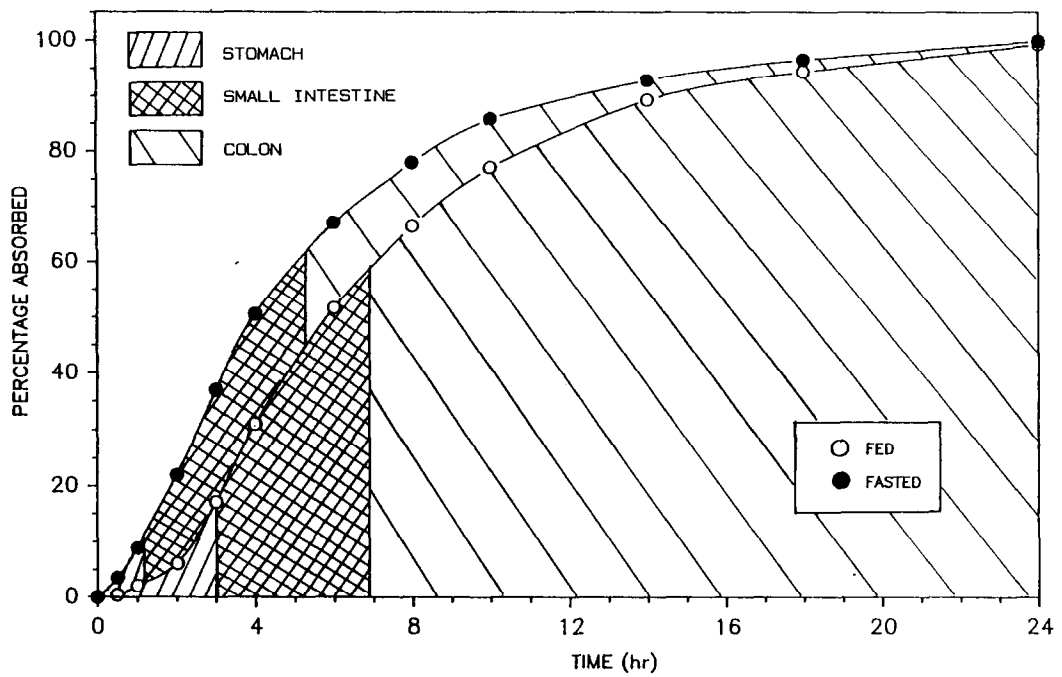

Fig. 5. Average percentage absorbed vs time plots of formula A dosed, fed and fasted, and distribution of pellets in the gastrointestinal tract.

effect on the transit time. Bryson et al. (1989) have investigated the effects of altering the small intestinal transit time on the absorption of a sustained release theophylline product. They reported that the bioavailability was not affected by a decrease in SITT. However, an increase in SITT resulted in a decrease in the rate of theophylline absorption, although the extent of bioavailability was unaffected.

A notable feature that can be observed in Fig. 5, as well as the individual absorption profiles (Figs 2 and 3) is that a considerable amount of drug absorption was occurring when the pellets were in the colon. This is noteworthy since the orocaecal transit time was relatively brief and the preparation was designed to release the drug over an extended time period. For both fed and fasted conditions, an estimated 40% of the drug was found to be absorbed when the pellets were in the colon, over a period of approx. 16 h (Fig. 5). This is in accord with the results obtained by De Sommers et al. (1990), in which approx. 40% of the administered dose of a sustained release theophylline product was reported to be additionally absorbed in the colon. Therefore, the theory that drug absorption could be limited to a definite segment of the gastrointestinal tract, or the so-called absorption window (Hirtz, 1984) does not apply to theophylline.

The extent of absorption when the pellets were in the different regions of the gastrointestinal tract was estimated from the individual absorption curves, and the numerical values are given in Table 3. The percentage absorbed whilst the pellets were in the stomach was estimated at $t_{50\%}$ gastric emptying, whilst that in the small intestine was estimated as the difference between $t_{50\%}$ gastric emptying and $t_{50\%}$ caecum arrival. The remaining amount constitutes the percentage absorbed in the colon. Only approx. 9% of the drug was absorbed during residence of the pellets in the stomach when fasted and 18% when fed. The latter increase could be attributed to the prolonged gastric residence time of the pellets. Comparable amounts were absorbed when the pellets were in the small intestine and colon, particularly in the fed mode. Thus, the colon constitutes an important absorption site for the oral sustained delivery of theophylline.

TABLE 3

Percentage theophylline absorbed during residence of pellets at various regions of the gastrointestinal tract

	Volunteer	Percentage absorbed		
		Stomach	Small intestine	Colon
Fasted	KI	2	58	40
	JE	4	52	44
	SL	3	72	25
	NA	2	71	27
	VK	23	25	52
	JA	17	46	37
	Average	9	54	37
Fed	LM	16	33	51
	KN	17	31	52
	EY	13	37	50
	AM	10	60	30
	WI	16	44	40
	WW	38	39	23
	Average	18	41	41
Overall average		14	47	39

The rates of absorption when the pellets were in each of the regions were approximated by dividing the percentage absorbed by the corresponding residence time, and are given in Table 4. Although this may be a crude approximation, it nevertheless permits the rate of drug availability to be compared, during the residence of the pellets in the different regions of stomach, small intestine and colon. The results were analyzed using an analysis of variance (ANOVA) procedure (split-plot), with the assumption that the data were obtained according to a two factorial split-plot design. In this case, the treatment levels of factor B would be the different regions of the gastrointestinal tract, and factor A the food status. The results of the statistical analysis shown in Table 4 indicate that the effects of food were not significant, nor was the interaction between the factors. This suggests that the absorption rate during residence of the pellets in each of the regions was not affected by food. On the other hand, a statistical significance was observed in factor B. A posteriori comparisons among the means using the Tukey's ratio (Kirk, 1968) revealed that the absorption rates when the pellets were in the three regions were significantly differ-

TABLE 4

Approximate rate of theophylline absorption (% h^{-1}) during residence of pellets at various regions of the gastrointestinal tract and anova results

		Volunteer	Factor B		
			Stomach	Small intestine	Colon
Factor A	Fasted	KI	10.9	15.3	2.0
		JE	2.7	10.3	2.5
		SL	7.5	13.6	1.4
		NA	15.0	18.0	1.4
		VK	8.9	12.0	2.7
		JA	7.5	9.6	2.2
		Average	8.8	13.1	2.0
Fed	LM	5.2	14.6	2.7	
		KN	5.7	8.0	3.0
		EY	4.3	10.8	2.8
		AM	5.1	12.2	1.8
		WI	5.5	9.5	2.4
		WW	9.3	9.1	1.5
		Average	5.9	10.7	2.4

ANOVA

Source	SS	df	MS	*F*	*P*
Factor A	25.0	1	25.0	3.07	0.108 (NS)
Error (m. plot)	81.5	10	8.1	–	–
Factor B	566.9	2	283.5	56.83	< 0.001
A×B	18.3	2	9.2	1.84	0.184 (NS)
Error (s. plot)	99.8	20	5.0	–	–
Total	791.7	35	–	–	–

ent and were of the following order (fastest to slowest): small intestine > stomach > colon.

Therefore, as expected from the individual absorption profiles, absorption was fastest when the pellets were in the small intestine, but because of the relatively brief residence time, it accounted for only approx. 40–50% of the drug to be absorbed. On the other hand, whilst absorption of the pellets in the colon was slow, this was compensated by a longer exposure of the pellets to this region. The slow absorption rate when the pellets were in the colon may in part be due to a lower drug concentration remaining in the dosage form. Since the drug release was basically a passive diffusion process, the rate of dissolution would thus be reduced. Other factors such as increased viscosity of the luminal contents or reduced motility, may further contribute to the slow absorption rate observed. In common with the colon, absorption from the pellets in the stomach was relatively slow compared to that in the small intestine and could be attributed to the small absorptive area. It may thus be adduced that, variations in residence time in this region was the main contributing factor for the differences observed in the serum profiles of the formulation between the fed and fasted conditions. Though this may be the case, the overall serum concentration profile remained unaffected, despite a slight delay or shift of the curve to the right, and may not be therapeutically significant.

Conclusion

The results obtained indicate that both gastric emptying and caecum arrival of the pellets were delayed when administered with food but the small intestinal transit time was not affected. In addition, the presence of food did not significantly alter the rate of theophylline absorption, although the delayed gastric emptying was associated with a slight delay in absorption, which may not be therapeutically significant. For both fed and fasted conditions, absorption of theophylline was fastest when the pellets were in the small intestine, followed by in the stomach and was slowest when in the colon. However, an appreciable amount of drug was absorbed in the colon, which thus acts as an important absorptive site for sustained release products of theophylline.

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