Studies on Freeze-Dried Drug–Milk Formulations II: Effect of Regenerated Fluid Volume on Nitrofurantoin Bioavailability

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Abstract □ The effect of different milk volumes on the extent and consistency of nitrofurantoin (1-[(5-nitrofurfurylidene)amino]hydantoin) absorption from freeze-dried nitrofurantoin–milk formulations was studied in four male volunteers in three separate crossover designs. Each volunteer received six single-dose treatments (one 100-mg nitrofurantoin capsule with 100, 200, and 400 mL of milk and 100 mg of nitrofurantoin as a freeze-dried nitrofurantoin milk formulation regenerated with 100, 200, and 400 mL of water). Analysis of the urine data revealed superiority of the nitrofurantoin–milk formulations regenerated with 200 and 400 mL of milk over the corresponding capsule formulations in the rates and extents of nitrofurantoin excretion. The binding of nitrofurantoin to casein and bovine serum albumin and its solubility in the presence of the proteins were measured in vitro. The presence of both proteins caused increases in the solubility of nitrofurantoin. Normal protein binding is responsible for the increase of nitrofurantoin solubility in the presence of bovine serum albumin, whereas the increase of nitrofurantoin solubility in the presence of casein is attributed to the formation of aggregates in casein solution at 37 °C. The in vivo data were discussed in light of the in vitro data. The freeze-dried nitrofurantoin–milk formulation regenerated with 200 mL of water has a potential for use as a nitrofurantoin delivery system.

A number of studies[1-7] have been concerned with the influence of fluid volume on the bioavailability of drugs administered in solid dosage forms. These studies have shown that the volume of co-administered fluid may significantly affect the rate and/or the extent of the absorption of sparingly water soluble drugs. On the other hand, the bioavailability of freely water soluble drugs is not affected by changing the fluid volume.

Recently, a novel drug delivery system utilizing milk as "inert vehicle" was proposed[8] as a means of enhancing the bioavailability of sparingly soluble drugs. The main feature of this system is that the drug–milk product is regenerated by adding water to the freeze-dried drug–milk formulation just prior to administration. Thus, a drug–milk product with most of the drug in solution is effectively ingested. One important factor which may affect the drug bioavailability from such a formulation is the potential influence of regenerated fluid volume on drug absorption. With the system proposed, most of the drug is already in solution when administered and therefore, concern arises about the dilution of this solution. Little data[9,10] are available with regard to whether or not the absorption of drugs is more complete from concentrated than dilute solutions. However, one must take some additional points into account when a freeze-dried formulation diluted to various degrees is ingested. First, stomach emptying can be affected by varying the volume of milk. Second, milk contains proteins which can decrease the absorption of drugs by formation of protein complexes. Since these protein–drug complexes are broken down by digestive enzymes, the overall absorption of drugs is dependent on the rate of these processes which are in turn related to the volume and type of milk. Third, the lipid content of milk can promote the solubility of sparingly soluble drugs but it can also delay the release of a drug with lipophilic properties. Again, the effect is related to the volume and type of milk used.

These considerations prompted us to examine the effect of the fluid volume used to regenerate the drug–milk formulations on bioavailability using nitrofurantoin (1-[(5-nitrofurfurylidene)amino]hydantoin) as a model drug. Thus, a study was conducted to compare the influence of milk volume on the extent and consistency of nitrofurantoin bioavailability from freeze-dried nitrofurantoin–milk formulations. Since nitrofurantoin is a compound that is poorly soluble in water[11,12] (0.3 mg/mL) and exhibits bioavailability problems,[13,14] the bioavailability of the freeze-dried formulations was also compared with a control capsule formulation. This was accomplished by administering regenerated nitrofurantoin–milk solutions of 100, 200, and 400 mL and a nitrofurantoin formulation in capsule form with 100, 200, and 400 mL of milk to healthy volunteers in three crossover designs.

Experimental Section

Dosage Forms—The method described previously[8] was employed to prepare the freeze-dried formulations. A volume of 20 mL of a 5 mg/mL nitrofurantoin (1-[(5-nitrofurfurylidene)amino]hydantoin; Sigma Chemicals, lot no. 43C–1550) solution in 0.1 M NaOH was added in a dropwise manner with stirring to the appropriate volume of low-fat milk (Long life Milk, fat concentration 0.75%, Landgenossenschaft, Ennstal, Stainach, Steiermark, Austria). Volumes of 100, 200, and 400 mL of milk were used. The pH of the final pale yellow solutions was checked and found to be altered in all cases by <0.4 pH units (pH range: 6.81–7.17). These solutions were further lyophilized (Secfroid, Lausanne, Switzerland), and the freeze-dried materials were collected and kept in airtight amber glass bottles.

A quantity of 100 mg of nitrofurantoin powder was placed in a size 00 gelatin capsule to make the control formulation. Size analysis (Coulter Counter, model TAII with a 400 μm apperture) of this powder showed a geometric mean diameter by weight of 74.81 μm, and a surface volume mean diameter by weight of 67.03 μm.

X-ray Diffraction Studies—Diffraction spectra (Philips X-ray diffraction unit type PW-1051) were prepared by scanning at a rate of 1 °/min in terms of a 2θ angle, from 2 ° to 40 °. Since the percentage of drugs in the freeze-dried formulations utilized for the bioavailability studies was too low [i.e., <0.51% (w/w)] a nitrofurantoin formulation of 13.5% (w/w) was prepared by the same method and used for the X-ray diffraction studies. Spectra were recorded for the pure drug substance, the physical mixture, and the freeze-dried formulation.

Protein Binding and Solubility Studies—The binding of nitrofurantoin was studied with casein (Serva Feinbiochemica, vitamin-free) and bovine serum albumin (BSA; Fluka A.G., fraction V) at 23 ± 0.5 °C by equilibrium dialysis using a Dianorm system (Bioblock Scientific) with 2-mL cells. All experiments were carried out in a 0.067 M, pH 7.4 phosphate buffer using protein concentrations of 0.05 and 1.0 mg/mL for casein and BSA, respectively. Nitrofurantoin was initially dissolved in dimethylformamide. The initial nitrofurantoin concentration ranged from 10 to 200 μg/mL.

The solubility of nitrofurantoin was measured either in the absence or presence of proteins. The study was carried out in 0.067 M

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phosphate buffer (pH 7.2). An amount in excess of nitrofurantoin was allowed to equilibrate while being shaken in an incubator (Julabo SW1). The system was held at 37 ± 0.1 °C after protection of the solutions from light.

Surface Tension Measurements—The surface tension of the protein solutions was measured in pH 7.4 (0.067 M) phosphate buffer with a du Noüy interfacial tensiometer coupled with a thermostat vessel (Kruss 8600) set at 20 ± 0.1 °C and 37 ± 0.1 °C. The solutions were equilibrated at the appropriate temperature for 1 h prior to the measurement.

Bioavailability Studies—The design for all studies was identical. Four healthy male volunteers, averaging 31 years of age (range 24–37 years) and weighing 69.2 kg (range 65–74 kg), participated in all studies. All subjects had normal screening vital signs and laboratory parameters. No subject received any other medication for 7 d preceding the start of the studies. All subjects received only the medication prescribed in the protocol for the duration of the study. The studies were separated by 1 month.

At zero time of each treatment period, each subject received single oral doses of medication as follows. Treatment A_{100}: one 100-mg gelatin capsule of nitrofurantoin (control formulation) with 100 mL of milk; or Treatment B_{100}: the regenerated nitrofurantoin–milk product that was prepared by adding 100 mL of water to the freeze-dried nitrofurantoin–milk formulation. Individual subjects were assigned treatments according to the standard two-period crossover design with a balance of treatments over periods. The washout period was 1 week.

The studies with Treatments A_{200}, B_{200}, A_{400}, and B_{400} were identical to the one described above but instead of using 100 mL, the volumes of fluid used were 200 and 400 mL, respectively. To counteract bitterness, 25 mg of saccharin sodium (Serva Feinbiochem, GMBH and Co.) was dissolved in the formulation prior to ingestion of all fluids.

Subjects were required to fast overnight and for 4 h immediately after the medication was administered. Urine samples were collected at zero time, i.e., just before dosing with each medication, and at 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, and 24.0 h. Complete emptying of the bladder was therefore ensured at each time point. The volume of each urine sample was recorded and the samples were kept frozen before analysis to ensure the stability of the drug. The samples were allowed to thaw immediately before use.

Assay—The amount of drug in urine was determined according to the method of Conklin and Hollifield.[15] The determination of nitrofurantoin in the in vitro samples was made by direct spectrometry at 380 nm after appropriate dilution. Bovine serum albumin (BSA) and casein did not interfere with the assay of nitrofurantoin. Calibration curves in the presence of either protein at the maximum concentrations utilized in the in vitro experiments were identical to those obtained in the absence of proteins. All estimations were made in duplicate.

Data Analysis—The graphically estimated pharmacokinetic parameters, i.e., percent of nitrofurantoin excreted in urine, peak nitrofurantoin excretion rate, and time of occurrence, were analyzed by an analysis of variance for crossover designs.

Results and Discussion

Preparation of Formulations and X-ray Studies—In all cases, no difficulties were encountered during the preparation of formulations and the regeneration of drug–milk products. All features of the methodology and the characteristics of the formulations were found to be identical to those reported previously.[8]

X-ray spectra of the drug–milk freeze-dried formulation, the physical mixture, and the pure drug substance are shown in Fig. 1. The two major diffraction peaks at 16.7 ° and 33.3 ° of nitrofurantoin are identified in the pure drug substance and the physical mixture sample. In contrast, both peaks can hardly be identified in the freeze-dried milk–drug sample. These findings combined with the observation of a rising base line (Fig. 1A and B) indicate that most of the drug has lost its initial crystalline form during the lyophilization process. In view of the high percentage of drug in the freeze-dried nitrofurantoin–milk sample used for the diffraction

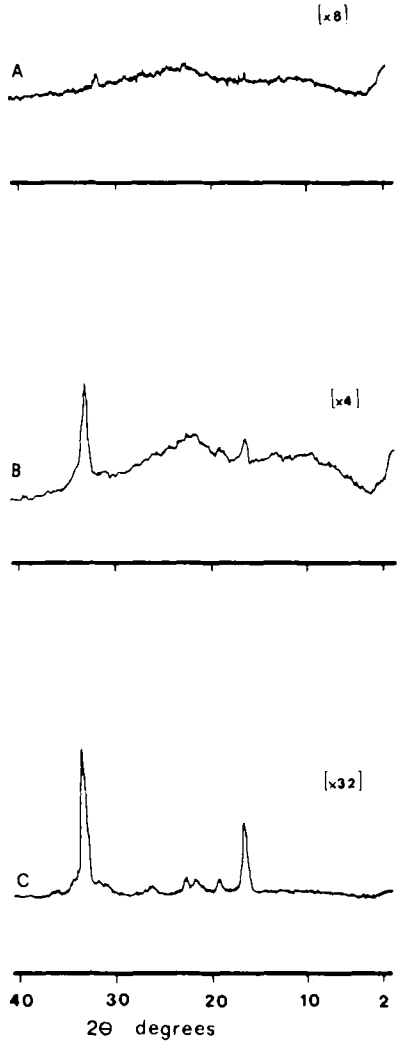

Figure 1—*Powder X-ray diffraction patterns for:* (A) *nitrofurantoin–milk freeze-dried system;* (B) *nitrofurantoin–milk physical mixture; and* (C) *nitrofurantoin. The values in parentheses correspond to the correction factors of different relative intensities of the reflections in each spectrum.*

studies, it can be concluded that nitrofurantoin is dispersed in the formulations used for Treatments B_{100}, B_{200}, and B_{400} in an amorphous state.

In Vitro Studies—In a previous article,[8] the high solubility of dicumarol in a casein solution was attributed to the formation of aggregates of casein. In order to explore this possibility in the present study, all in vitro experiments were carried out with two proteins, BSA and casein. It was felt that the comparable studies would allow the elucidation of the mechanism of the effect of casein on nitrofurantoin solubility.

The solubility studies were performed at pH 7.2 to mimic the pH of the final drug–milk solutions, while the protein binding was studied at pH 7.4. Based on the plausible assumption that this pH difference does not significantly affect the conformation of the proteins, correlations of solubility with protein binding results were made.

Figure 2 graphically presents the result of the surface tension measurements for both proteins. While the plot of surface tension versus the log of BSA concentration is linear at both temperatures studied, the plot of casein at 37 °C has two distinct phases. The surface tension drops as casein concentration increases and remains practically constant above a casein concentration of ~0.004 mg/mL. This concen-

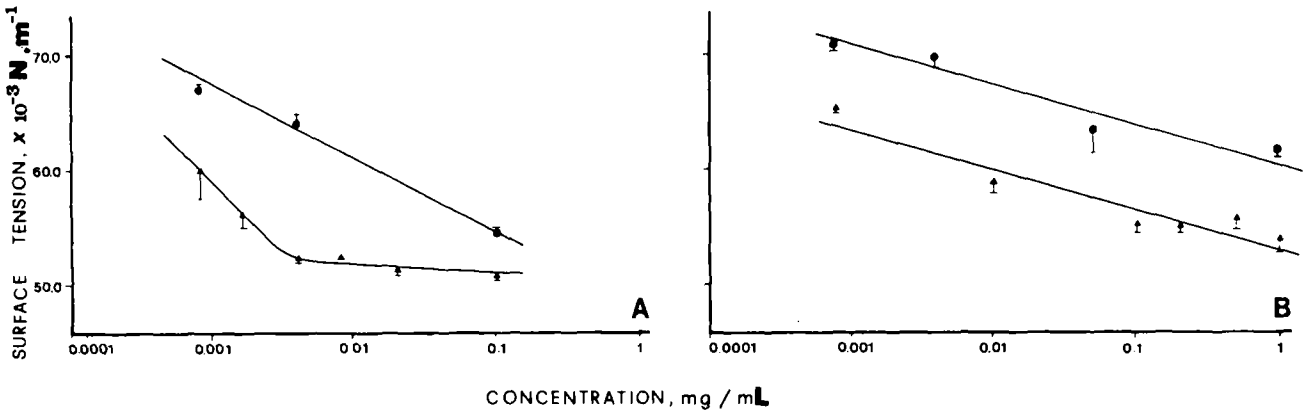

Figure 2—*Surface tension of casein* (A) *and BSA* (B) *solutions in pH 7.4 (0.067 M) phosphate buffer as a function of protein concentration. Each point is the mean ± SD of four determinations. The equilibration time was 1 h in all cases. Key:* (●) *20°;* (▲) *37°.*

tration range corresponds to the region of the critical micelle concentration where formation of casein aggregates becomes appreciable.[16,17]

Experimental results in percentage of drug bound to protein, obtained from equilibrium dialysis experiments for the binding of nitrofurantoin to 1.0 mg/mL of BSA and 0.05 mg/mL of casein, are given in Fig. 3. The decrease in percent binding of nitrofurantoin as its concentration increases is indicative of specific saturable binding of nitrofurantoin:BSA and nitrofurantoin:casein interactions.

The solubility of nitrofurantoin at 37 °C was increased in the presence of both proteins (Fig. 4). The solubility values found in the presence of proteins can be discussed in light of the surface tension and protein binding findings. The increase of nitrofurantoin solubility in the presence of BSA is most likely due to protein binding since the effect has a linear character and the amount of nitrofurantoin bound, given by the increase in solubility above the intrinsic solubility, is in agreement with the binding observed using a BSA concentration of 1 mg/mL (Fig. 3). On the other hand, the effect of casein on the solubility of nitrofurantoin is much higher than can be accounted for by normal binding. Besides, Fig. 4A is a typical solubility curve for a surface active agent, solute, and solvent system.[18] This observation is in accordance with Fig. 2 where the region of casein aggregates formation is nicely delineated. It can be concluded, therefore, that nitrofurantoin is solubilized by casein aggregates.

In Vivo Studies—Figure 5 graphically presents the cumulative amount of nitrofurantoin recovered as unchanged drug in urine versus time for the six treatments. From this figure, the percentages of total drug excreted were estimated and the values found are quoted in Table I, along with the peak excretion rate, Q_{max}, and the peak excretion time, t_{max}, calculated graphically from an excretion rate versus time plot. Analysis of variance was performed on the three parameters estimated comparing the treatments utilizing the same milk volume. The results obtained are summarized in Table II. Significant differences ($p \leq 0.05$) in drug parameters for the formulations examined were found. The freeze-dried formulations prepared with 200 and 400 mL of milk were significantly different from the corresponding capsule formulations for all parameters calculated. The freeze-dried formulation prepared with 100 mL of milk was found to be equivalent to the corresponding capsule formulation in terms

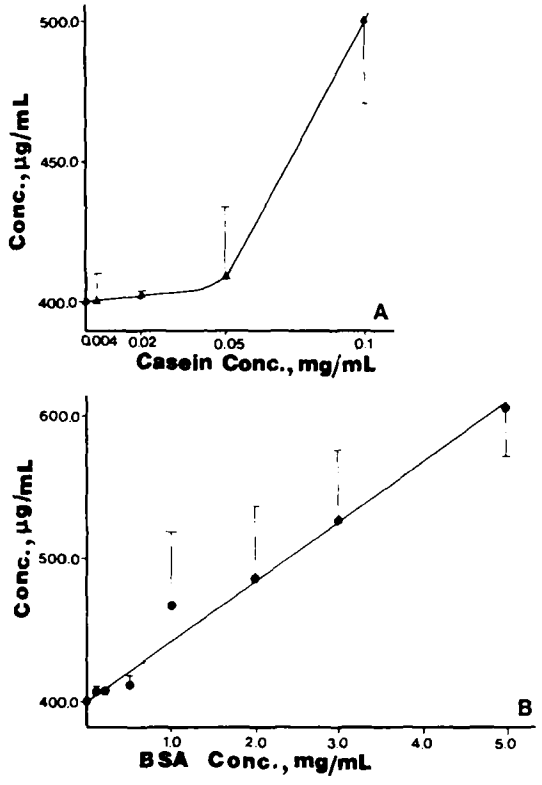

Figure 4—*Solubility of nitrofurantoin as a function of casein* (A) *and BSA* (B) *concentration. Each point is the mean ± SD of four determinations.*

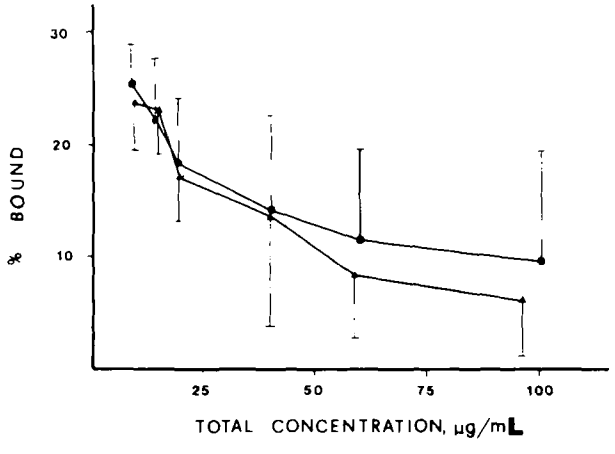

Figure 3—*Percentage of nitrofurantoin bound to casein* (●) *and BSA* (▲) *versus total drug concentration. Each point is the mean ± SD of three determinations.*

of the total amount of nitrofurantoin excreted. It is interesting to note that a comparison between all pairs of the freeze-dried formulations did not reveal significant differences for all parameters estimated. Based on all parameters calculated, there were also no significant differences found between capsules co-administered with varying milk volumes (100 versus 200 mL, 100 versus 400 mL, and 200 versus 400 mL).

Figure 6 illustrates the between-subject variability in cumulative urinary excretion of nitrofurantoin for the treatment. The CV values ranged from 50.6 to 60.3% for capsules and from 13.2 to 25.6% for the freeze-dried formulations. Obviously, the freeze-dried formulations exhibit dramatically smaller CV values than the capsules for all volumes of milk utilized.

Previous studies[19,20] have shown that the bioavailability of nitrofurantoin is promoted by the presence of food. This has been attributed to the delay of gastric emptying which results in an increase of the residence time in the stomach. It has also been reported[21] that the small intestine is the major site of absorption of nitrofurantoin. Consequently, the influence of the volume of milk taken and the change of gastric emptying rate could affect the bioavailability of nitrofurantoin. It has been suggested[22] that increased fluid volumes can enhance the absorption of some drugs, particularly those with long dissolution times. On the other hand, large fluid volumes could reduce the absorption of drugs that are readily soluble, probably due to a decreased concentration gradient across the gastrointestinal wall.[2] However, neither of these suggestions would ideally apply to the results obtained in the present study since the rate and extent of bioavailability were found to be independent of the fluid volume.

The decreased and delayed absorption (peak excretion time of 3 or 4 h) of nitrofurantoin from capsules can possibly be explained in terms of the slow dissolution of the drug in milk under the in vivo conditions. This argument is substantiated by the fact that the lag time caused by the capsule itself is insignificant as was demonstrated[8] by the results obtained with the more water soluble sulfamethizole using an identical capsule formulation. In parallel, it has recently been shown[23,24] that the rate of dissolution of drugs in milk from conventional and controlled-release formulations is generally slower. In addition, the results with milk products (Table I) show that regardless of the milk volume, rapid gastric emptying has occurred. Fast stomach emptying would increase the rate at which drug particles from the capsule formulation pass the small intestine and, hence, reduce drug bioavailability.

The increase of the milk volume from 100 to 200 mL in the

Table I—Values of the Bioavailability Parameters of Nitrofurantoin Formulations[a]

Bioavailability Parameter	Treatment					
	A_{100}	B_{100}	A_{200}	B_{200}	A_{400}	B_{400}
F, %[b]	26.33 (14.72)	38.82 (9.96)	27.61 (10.43)	50.19 (9.29)	21.21 (12.79)	41.26 (5.43)
Q_{max}, mg·h^{-1}[c]	6.06 (2.89)	12.40 (2.54)	5.34 (1.86)	18.55 (3.72)	5.28 (3.09)	15.33 (4.09)
t_{max}, h[d]	3.0 (0.8)	1.5 (0.6)	4.0 (1.4)	1.5 (0.6)	3.0 (0.8)	1.5 (0.6)

[a] Mean values of four subjects with standard deviation in parentheses. [b] Percentage of total drug excreted. [c] Peak excretion rate. [d] Peak excretion time.

Table II—Significant Differences in Drug Parameters for the Formulations Studied

Bioavailability Parameter	Treatment		
	A_{100} and B_{100}	A_{200} and B_{200}	A_{400} and B_{400}
F, %[a]	N.S.[b]	0.01	0.05
Q_{max}, mg·h^{-1}[c]	0.05	0.01	0.01
t_{max}, h[d]	0.05	0.05	0.05

[a] Percentage of total drug excreted [b] Not significant at the 0.05 level. [c] Peak excretion rate. [d] Peak excretion time.

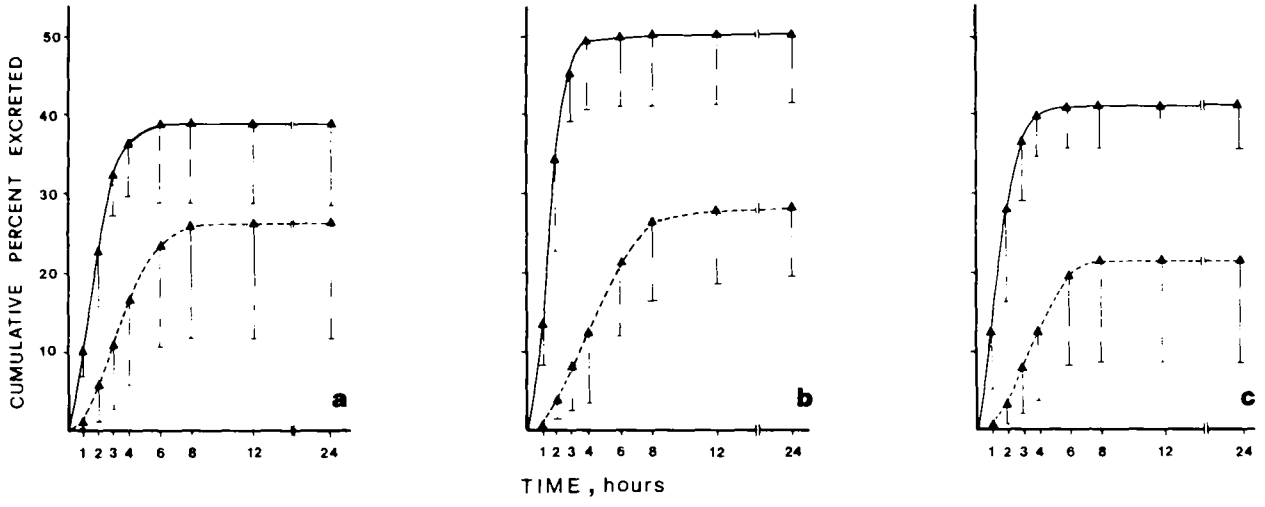

Figure 5—*Mean cumulative percent of nitrofurantoin excreted following the administration of a freeze-dried formulation (——) or a capsule (– – – –). Each point is the mean ± SD of the cumulative percent excreted for all four subjects. Volume of milk used:* (a) *100 mL;* (b) *200 mL;* (c) *400 mL.*

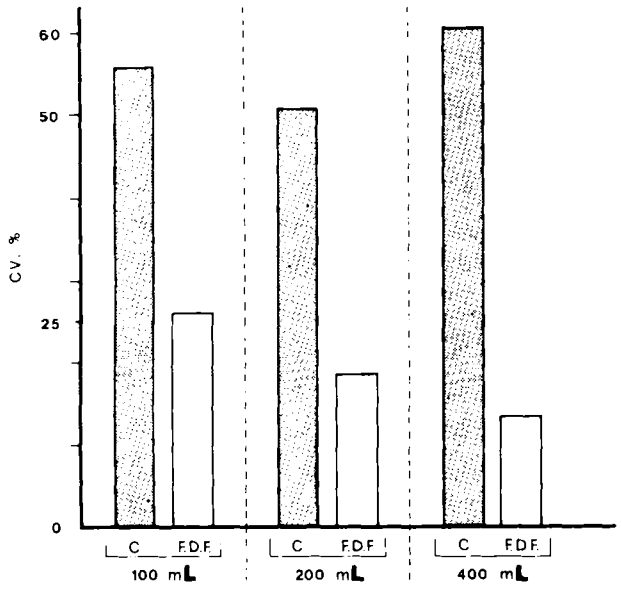

Figure 6—*Between-subject variability (expressed as CV) in cumulative urinary excretion of nitrofurantoin for capsules* (C) *and freeze-dried formulations* (F.D.F.).

regenerated milk products tended to improve the bioavailability of nitrofurantoin, although the differences were not statistically significant. A further increase to 400 mL of milk resulted in lower bioavailability, possibly because of a decreased concentration gradient across the GI barrier, but again, the difference was not statistically significant. Considering the results in their entirety it seems likely that in the range of 100 to 400 mL of milk, the rate of gastric emptying does not change appreciably. In agreement with this conclusion is the identical t_{max} values found for the milk products (Table I). Contrasting the literature data[19,20] on the effect of food on nitrofurantoin bioavailability with the results of this study, we can also conclude that gastric emptying is much slower in the presence of food than when 100 to 400 mL of milk is administered. This observation coincides with the effect of composition of a meal on the rate of gastric emptying.[25,26]

The results of this study reveal superiority of the freeze-dried formulations over the control capsules (except 100 mL versus 100 mL) with respect to the total amount of nitrofurantoin excreted, the rate parameters, and the smaller interindividual variation in the amount of total nitrofurantoin excreted. Moreover, it is worthy of mention that nausea was encountered in two cases 2 h after capsule ingestion. The freeze-dried formulations did not induce nausea. The regenerated 200- and 400-mL freeze-dried formulations probably owe their superiority to their ability to maintain most of nitrofurantoin in solution at the absorption sites. This is well substantiated by the in vitro results (Fig. 4). It should be pointed out, however, that due to solubility limitations of casein, the solubility studies of nitrofurantoin were performed in casein solutions where the protein concentration was $\leq$0.1 mg/mL. Because of the complexity of the structure of milk, explicit quantitative extrapolations of the in vitro solubility values to nitrofurantoin solubility in milk, where the casein concentration is ~25 mg/mL, cannot be made. Nevertheless, it is reasonable to argue that the qualitative character of the effect of casein in milk on nitrofurantoin solubility remains the same. Therefore, the overall effect of the enhanced solubility of nitrofurantoin in the drug–milk formulations administered is that the absorption of drug (as reflected in the percentages of nitrofurantoin excreted) is more rapid and complete than its absorption from the capsule formulations. The better absorption of drug from the drug–milk solutions was also demonstrated by the low interindividual variation in nitrofurantoin bioavailability (Fig. 6). The latter characteristic of the freeze-dried formulations regenerated with 200 and 400 mL of water, could be attributed to the avoidance of dissolution related factors which cause erratic absorption of drug from the capsule formulations (Fig. 6). The equivalence noted between Treatments A_{100} and B_{100} in terms of total amount of nitrofurantoin excreted cannot be explained with any degree of certainty. Probably it arises from a variety of reasons such as (*a*) limited ability of the 100 mL of milk to keep the drug in solution under the gastrointestinal conditions; as a result of this, (*b*) partial precipitation from the drug–milk solution during mixing could happen; and (*c*) the small fluid volume utilized (100 mL) may tend to be emptied from the stomach at a slow rate. This, together with the influence of digestive processes, gives rise to less reproducible drug absorption.

The results of this study indicate that the volume of milk utilized can have a marked influence on the consistency of nitrofurantoin absorption from the formulations studied. It can be concluded that the formulation prepared by adding 200 mL of water to the freeze-dried nitrofurantoin–milk system is the most advantageous since (*a*) it showed the most intense statistically significant differences from the corresponding capsule formulation for all bioavailability parameters studied; (*b*) it demonstrated more consistent absorption, validated by a reasonably low coefficient of variation in the total amount of nitrofurantoin excreted; and (*c*) it offers convenience of administration in comparison with the formulation with the large fluid volume of 400 mL.

Such pharmaceutical information is useful in identifying factors which may or may not influence the absorption of drugs from the freeze-dried drug–milk formulations. This information can be further used for the appropriate design of these formulations.

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