Studies on Drug–Milk Freeze-Dried Formulations I: Bioavailability of Sulfamethizole and Dicumarol Formulations

PANAYOTIS E. MACHERAS[x] AND CHRISTOS I. REPPAS

Received November 18, 1985, from the *Department of Pharmacy, University of Athens, Athens 10680 Greece.* Accepted for publication April 14, 1986.

Abstract □ In this study, solid dispersion formulations of dicumarol (3,3'-methylenebis[4-hydroxycoumarin]) and sulfamethizole (*N'*-(5-methyl-1,3,4-thiadiazol-2-yl)sulfanilamide) in defatted milk were prepared by freeze-drying. X-ray crystallographic data showed that both drugs were dispersed in the formulations in an amorphous state. Bioequivalency comparisons between freeze-dried formulations, after regeneration with water, and control capsules containing the pure drug substances were studied in four male volunteers. Determination of the plasma dicumarol levels indicated superiority of the dicumarol-milk formulation. Statistically significant differences were found between area under the curve, maximum plasma concentration, and apparent elimination rates. Analysis of the urine sulfamethizole data revealed that the two formulations exhibit statistically equivalent rates and extents of excretion of unchanged sulfamethizole. The binding of both drugs to casein and their solubility in the presence of casein were measured in vitro. The presence of casein caused an increase in the solubility of dicumarol, while it had no effect on the solubility of sulfamethizole. Normal protein binding cannot be responsible for the effects noted. Extrapolation of the in vitro data to the in vivo situation was attempted. Drug–milk freeze-dried formulations are promising for the enhancement of the bioavailability of sparingly water soluble drugs.

Although the oral route is most frequently used for introducing drugs into the body, it should be recognized that this mode of administration may often result in inefficient and erratic drug therapy. Several factors are interposed between administration of the dose and the appearance of drug in the blood. These factors can be broadly divided in terms of: (*a*) the physicochemical properties of the drug and the type of formulation; and (*b*) the various interactions between the drug, the formulation, and the components of the gastrointestinal (GI) tract.

Among the more important aspects associated with the rate and extent of absorption of an orally administered solid dosage form are the dissolution characteristics of the drug. Of all the possible manipulations of the physical properties of sparingly water soluble drugs to yield better absorption, the use of finely subdivided or micronized particles,[1–4] and the preparation of drug–polymer dispersions[5–7] by fusion and solvent techniques are the most widely exploited.[8–14]

Apart from the physicochemical properties of the drug and the type of formulation, the oral absorption of a drug is also influenced by the endogeneous and exogeneous components of the GI tract. Provided that gastrointestinal disease is not encountered, the relatively standard in vivo process of absorption involves endogeneous factors such as gastrointestinal secretions, intestinal motility, variable pH, and biotransformation. In contrast, exogeneous factors, such as food intake, vary considerably and can markedly influence the bioavailability of a drug. In recent years, much emphasis has been placed on whether or not drugs should be co-administered with a meal.[15–18] Food and other substances present in the diet can either increase or decrease drug absorption.[19] The fluid volume taken with a drug can also have a pronounced effect on the bioavailability of the drug.[20–21]

The considerable evidence that drug bioavailability is influenced by food, in conjunction with the poor and erratic bioavailability of sparingly soluble drugs, serve as stimuli for examining new formulations that may provide more consistent absorption. The present investigation reports the preparation and bioavailability of drug–milk freeze-dried formulations. Milk is employed as an "inert vehicle" since: (*a*) it is a convenient daily ritual with which to associate the administration of drugs; (*b*) it is a fluid in which drugs may be distributed within different phases (both the aqueous and the lipid phases may act as solvents, and adsorption on proteins is also possible[22]); and (*c*) it has a strong buffering capacity. Dicumarol and sulfamethizole were chosen as model drugs for this study. The former is a very sparingly soluble drug and its bioavailability is profoundly affected by concomitant food intake,[23] while the latter does not present serious bioavailability problems.[24] It was felt that the diversity of the solubility properties[25,26] of the two drugs would enable correlations to be made with the in vivo results.

Experimental Section

Test formulations—Freeze-drying was employed to make the test formulations. The same method was used for both drugs. A quantity equal to a single dose, i.e., 300 mg for dicumarol and 500 mg for sulfamethizole, was dissolved in 0.1 M NaOH. The resulting solution (~20 mL) was added in a dropwise manner to 200 mL of defatted milk (Long Life Milk, fat concentration 0.75%, Landgenossenschaft, Ennstal, Stainach, Steiermark, Austria) with constant magnetic stirring and continuous pH monitoring. The difference in pH values between the pure milk and the final drug–milk solution was <0.5 pH units (pH range: 6.81–7.28) for both solutions. The drug–milk solution was poured into a disk of the freeze dryer (Secfroid, Lausanne, Switzerland) and lyophilized. The solid freeze-dried material was collected and kept in an air-tight amber glass bottle (formulation A).

The pure drug substances (dicumarol from Sigma; sulfamethizole from Cyanamid) were used for preparation of the control capsule formulations. Size 00 gelatin capsules were used. A quantity equal to a single dose, i.e., 300 mg for dicumarol and 500 mg for sulfamethizole, was placed in one or two capsules, respectively (formulation B).

Particle Size Analysis—The same batch of each drug was used to make formulations A and B. The drug powders were checked (Coulter Counter model TA II with a 400-μm aperture) for their particle size distribution. The geometric mean diameters by weight for dicumarol and sulfamethizole were 48.67 and 29.22 μm, respectively. The surface volume mean diameter by weight of dicumarol was 42.82 μm, whereas that for sulfamethizole was 28.29 μm.

X-ray Diffraction—Powder X-ray diffractometry was carried out employing cobalt radiation (Philips, PW-1051). Since the percentage of drugs in formulation A utilized for the bioavailability studies was too low, i.e., 1.51% and 2.51% (w/w) for dicumarol and sulfamethizole, respectively, formulations with higher drug content were made and used for the X-ray diffraction studies. These were made by the aforementioned method, but the percentage of drugs was 13.4% (w/

0022-3549/86/0700-0692$01.00/0

w). Spectra were recorded for the pure drug substances, pure freeze-dried milk, physical mixtures of drugs with freeze-dried milk, and the freeze-dried formulations of both drugs. They were prepared by scanning at a rate of 1 °/min, in terms of a 2θ angle, from 2 to 40 °

Protein-Binding and Solubility Studies—The binding of both drugs with casein (Serva Feinbiochemica; vitamin-free) was studied at 23 ± 0.5 °C by equilibrium dialysis using a Dianorm system (Bioblock Scientific) with 2-mL cells. All experiments were carried out in 0.067 M pH 7.4 phosphate buffer using a protein concentration of 0.05 mg/mL. Both drugs were initially dissolved in weakly alkaline solutions. The initial concentration of drugs ranged from 3 to 30 μg/mL and 5 to 150 μg/mL for dicumarol and sulfamethizole, respectively.

The solubilities of the two drugs were measured either in the absence or presence of casein. The study was carried out in 0.067 M phosphate buffer at pH 7.5 for dicumarol and pH 7.2 for sulfamethizole. An amount in excess of either drug 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.

Bioavailability Studies—Four healthy male volunteers participated in both studies. Their age and weight ranges were 23–35 years and 66–72 kg, respectively. Each subject received extensive information about the study and gave written consent. All subjects refrained from alcohol and any drug for at least 48 h before drug administration, as well as during the investigation. Before the day of drug ingestion, the volunteers abstained from all food and liquid for 10 h. Individual subjects were assigned formulations according to the standard two period crossover design with a balance of formulations over periods. Washout periods for sulfamethizole and dicumarol were 2 weeks and one month, respectively. The dicumarol study was initiated three months after the completion of the sulfamethizole study.

Dicumarol—Before administration, the liquid drug–milk solution was regenerated by adding 200 mL of water to the freeze-dried dicumarol-milk formulation. Each volunteer received either the regenerated dicumarol-milk solution (formulation A) or a capsule of 300-mg dicumarol (formulation B) with 200 mL of defatted milk. Due to the bitter taste of dicumarol 25.0 mg of saccharin sodium (Serva Feinbiochem. GMBH and Co.) were dissolved in both fluids before ingestion. All subjects continued to fast for an additional 4 h after the administration of the formulations. Twenty-four hours after taking dicumarol, each subject received 20-mg vitamin K (Konakion, Hoffmann-La Roche, Basle, Switzerland) orally to counteract the anticoagulant effect of dicumarol. Blood samples (4 mL) were obtained by means of a butterfly needle (21 INT, Abbott Lab.) during the first 8 h. Blood samples were collected at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 48.0, and 72.0 h after taking the formulations. Plasma samples were stored for <2 d at −30 °C before they were measured. The exact time of blood sampling was noted and used in calculations and graphs.

Sulfamethizole—Before administration, the liquid drug–milk solution was regenerated by adding 200 mL of water to the freeze-dried sulfamethizole-milk formulation. Each volunteer received either the regenerated sulfamethizole-milk solution (formulation A) or two capsules, each containing 250 mg of sulfamethizole (formulation B), with 200 mL of defatted milk. To counteract bitterness 25.0 mg of saccharin sodium was dissolved in both fluids prior to ingestion. All subjects continued to fast for an additional 4 h following administration of the drug. Urine samples were collected at 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, and 24 h after taking the medication, and were stored in the refrigerator for not more than one day before assay.

Assay—The plasma concentration of dicumarol was determined by the method of Nagashima et al.[27] Unchanged and total sulfamethizole in urine were estimated by the Bratton–Marshall method.[28] The same methods were used for the assay of drugs in the in vitro studies. All estimations for both drugs were made in duplicate.

Data Processing—Apparent half-lives of dicumarol elimination were calculated by least-squares regression analysis of the terminal linear phase of the log concentration–time curves. Apparent excretion rate constants of unchanged sulfamethizole were calculated by linear regression analysis of the terminal linear phase of log cumulative amount excreted–time curves.[29] Areas under the plasma dicumarol concentration–time curves were calculated by the trapezoidal rule. Areas under the plasma dicumarol concentration–time curves, peak dicumarol plasma concentrations and times of occurrence, percent of unchanged sulfamethizole excreted in urine, peak sulfamethizole excretion rates and times of occurrence, apparent half-lives of dicumarol elimination, and the unchanged excretion rate constants of sulfamethizole were analyzed by an analysis of variance for crossover designs. Total variance was separated into that due to subjects, day of administration, formulation, and residual.

Results and Discussion

Preparation of Formulations and X-ray Studies—Defatted milk was used since freeze-drying of whole milk could be problematic due to the presence of large amounts of free fat in the dried product, implying a rather poor "keeping quality" of the freeze-dried milk.[30] The preparation of the milk–drug solutions carried out at room temperature did not necessitate special conditions. The slight change in pH of the drug–milk solution during the preparation and the short stay of the resulting solution under the slightly alkaline conditions cannot be considered harmful. Consequently, any destruction of milk components can be completely ruled out. The homogeneity of the solutions was not considered since each solution contained a single dose and was lyophilized immediately after preparation. However, visually clear, homogeneous liquids were obtained with both drugs. Difficulties were not encountered in regenerating the milk–drug solutions just prior to administration. Mechanical agitation with a spoon for 1–2 min was found to be adequate to obtain homogenous liquids.

X-ray spectra of freeze-dried milk, pure drug substances, physical mixtures, and drug–milk freeze-dried formulations are shown in Fig. 1. The dicumarol and sulfamethizole diffraction peaks (14.0, 17.7, 29.0, and 32.4 °, and 9.7, 14.0, 23.6, and 32.4 °, respectively) are identified in the pure drug substances and the physical mixtures samples. None of these peaks can be identified in the freeze-dried milk–drug sam-

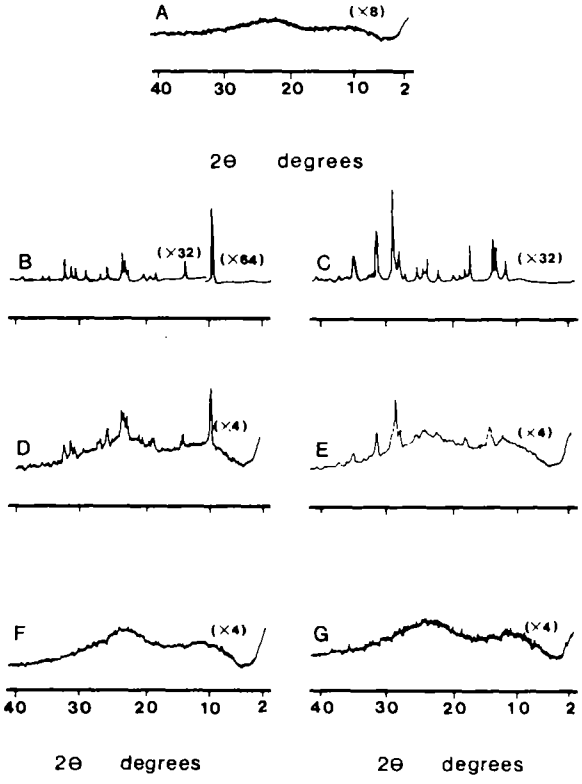

Figure 1—*Powder X-ray diffraction patterns for: (A) freeze-dried milk; (B) sulfamethizole; (C) dicumarol; (D) sulfamethizole-milk physical mixture; (E) dicumarol-milk physical mixture; (F) sulfamethizole-milk freeze-dried system; and (G) dicumarol-milk freeze-dried system. The values in parentheses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

ples. These findings, combined with the observation of a rising base line (Fig. 1, F and G), indicate a complete loss of the initial crystalline form of the two drugs during the lyophilization process. It can be concluded, therefore, that both drugs are dispersed in the formulations in an amorphous state.

Protein-Binding and Solubility Studies—Since casein is the main protein in cow's milk,[31] protein-binding and solubility studies were performed to evaluate the extent of the interaction of two drugs with casein. The results of the binding experiments, in percentage of drug bound to casein, are given in Fig. 2. For both drugs, the degree of casein binding decreased with increasing drug concentration. As seen in Fig. 2, both drugs are minimally bound (<13%) to casein over the ranges of drug concentrations utilized (solubility limitations for dicumarol did not allow a wider range of concentrations).

Since casein forms micelles in milk, solubility experiments were carried out at 37 °C to allow for the formation of micelles in the aqueous solution.[31] The effect of casein on the saturation solubility of dicumarol is shown in Fig. 3. Higher casein concentrations were not used due to solubility limitations. The presence of casein caused a significant increase in the solubility of dicumarol. The main characteristic is that solubility increases, with an increase in added protein, until a plateau level is reached. The maximum enhancement was ~110% and occurred when casein was at a level of 0.05 mg/mL; a value which is above the concentration for critical micelle formation.[32] Since the effect has a nonlinear character, the increase of dicumarol solubility with protein concentration cannot be attributed to normal protein binding. Moreover, the binding of dicumarol to casein was shown to be much too small (Fig. 2) to account for the observed increase in solubility.

The sulfamethizole solubility in the absence of casein was equal to 10.36 ± 0.10 mg/mL. At pH 7.2, for casein concentrations ranging from 0.01–0.1 mg/mL, no effect on sulfamethizole solubility was observed.

It is highly probable that the formation of aggregates in casein solution at 37 °C is the predominant factor for the solubility effects noted.[31] The dissimilarity of the influence of casein on the solubility of two drugs is probably linked with the vast difference in lipophilicity of the two drugs: i.e., the log P for dicumarol in an octanol:water system is equal to 2.07,[33] while log P values for sulfamethizole vary from −1.82 to 0.54.[34–36] Being hydrophobic, the dicumarol molecules can be dissolved inside casein micelles: i.e, they are brought into solution in an overall aqueous medium. Obviously, this is not applicable to the more hydrophilic sulfamethizole.

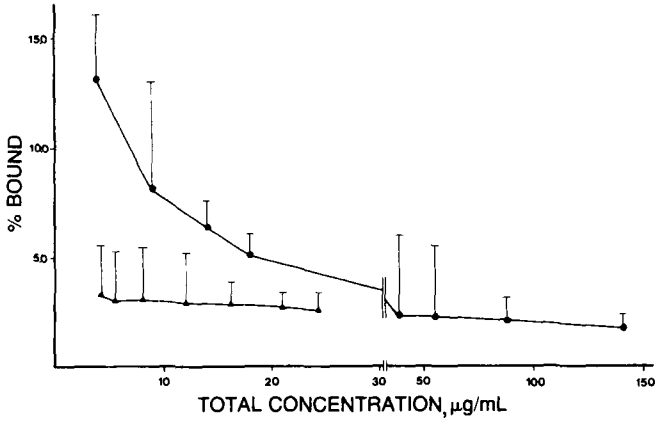

Figure 2—*Percentage of dicumarol (▲) and sulfamethizole (●) bound to casein versus total drug concentration. Each point is the mean ± SD of three determinations.*

Comparison of Dicumarol Treatments—Figure 4 graphically depicts dicumarol plasma concentration–time data in four subjects following the two oral treatments. In each of the four subjects, the formulation A curve differed dramatically from the formulation B curve. Calculations on paired observations revealed statistically significant differences between formulation A and formulation B peak concentrations, area under plasma concentration curve (AUC) values, and apparent elimination half-lives (Table I). As the absorption of dicumarol is prolonged, the absorption and elimination phases cannot be safely discriminated, and the conventional calculation of absorption rates was not done. Nevertheless, estimation of partial AUC values indicated that, from 0–4 h, the mean extent of absorption was 495% greater for the formulation A curve than for the control capsule curve. It is most likely that no precipitation of dicumarol took place in the GI tract after the ingestion of formulation A, and hence dissolution was bypassed. Apparently, formulation A delivers the drug in solution to the absorption sites. Thus, factors associated with the dissolution characteristics of dicumarol, causing limited and erratic absorption,[37] did not operate. In light of the in vitro solubility results, it is likely that the milk components increase the saturation solubility of dicumarol and hence, the concentration gradient which controls the rate of transfer. In addition to that, the dicumarol molecules

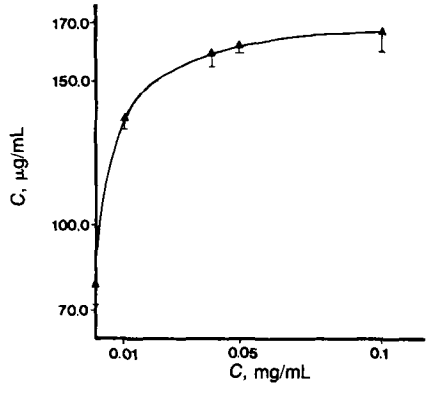

Figure 3—*Solubility of dicumarol as a function of casein concentration. Each point is the mean ± SD of four determinations.*

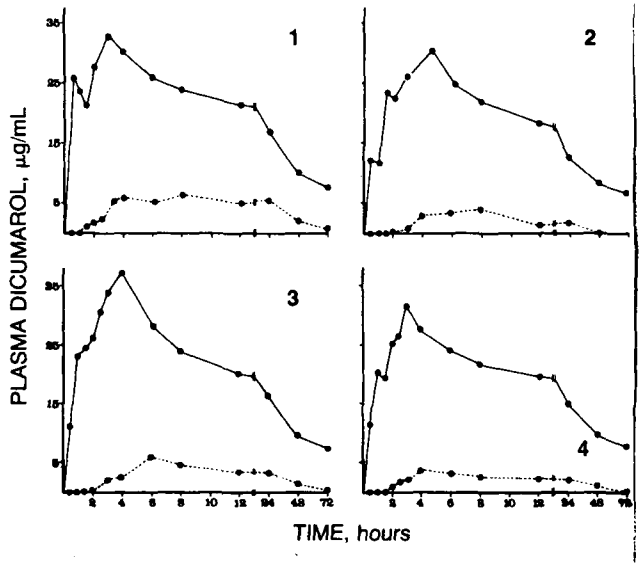

Figure 4—*The individual plasma concentrations of dicumarol in subjects 1–4. Key: (——) formulation A; (– – –) formulation B.*

Table I—In Vivo Results for Two Dicumarol Formulations Administered to Four Subjects

	Subject	C_{max}, μg/mL	t_{max}, h	$AUC_{0\to4}$, μg · h · mL^{-1}	$AUC_{0\to72}$, μg · h · mL^{-1}	$t_{1/2}^{a}$, h
Formulation A	1	33.0	3.0	102.0	1055.8	94.9
	2	30.1	4.7	80.3	881.9	120.9
	3	37.5	4.0	101.4	1044.7	96.7
	4	31.5	3.0	88.3	981.5	114.7
		(33.0)[b]	(3.7)	(93.0)	(991.0)	(106.8)
Formulation B	1	6.3	8.0	9.1	234.3	32.1
	2	3.8	8.0	2.0	65.2	18.2
	3	5.8	6.0	3.3	153.0	27.0
	4	3.6	4.0	4.4	103.7	24.8
		(4.9)	(6.5)	(4.7)	(139.0)	(25.5)
Levels of significance[c]		$p < 0.01$	NS	$p < 0.01$	$p < 0.01$	$p < 0.01$

[a] Calculated by linearization of the elimination phase using the last three data points. Values of correlation coefficients ranged from −0.963 to −0.996. [b] Averages in parentheses. [c] Probability of equivalence as determined by ANOVA. NS at $p = 0.05$ level.

in solution have the characteristics required for crossing the lipid membranes, as is exemplified by their high log P values. It is conceivable that dicumarol absorption cannot be retarded as a result of protein binding since milk digestion causes a progressive release of the free form of the drug in the GI fluids. Similar conclusions have been derived from in vitro studies focusing on the effect of protein on the dissolution and absorption of phenytoin.[38,39]

Interindividual variation in AUC values was reduced from almost 3.6-fold for formulation B to <1.2-fold for formulation A. It is worth mentioning that the greatest enhancing influence on dicumarol absorption was noted with subjects 2 and 4 who had the most incomplete absorption of the drug following administration of formulation B. This observation is in agreement with previous results.[23] Overall, though, contrary to previous findings,[23,37] the absorption of dicumarol from formulation A was found to be consistent, as is demonstrated by the low interindividual variation of AUC values.

The control capsule formulation exhibited dramatically low bioavailability accompanied by high lag times (Fig. 4). These results are probably attributable to the limited dissolution of dicumarol and not to that of the gelatin capsule itself. The latter alternative should be completely ruled out in view of the results obtained with the sulfamethizole capsule formulation (Fig. 5). Differences observed between the elimination half-lives are in accordance with the well known fact that the apparent first-order rate constant for dicumarol elimination decreases when the amount of drug reaching the general circulation increases.[40]

Comparison of Sulfamethizole Treatments—The average cumulative amount of unchanged and "total" sulfamethizole excreted versus time is shown in Fig. 5. The ranges of the percentage of sulfamethizole excreted as acetylated metabolite were 7.0–16.6% and 9.0–15.9% for formulations A and B, respectively. These values are within the ranges reported previously.[24] Mean excretion rate profiles of free sulfamethizole are shown in Fig. 6. Differences in the extent of bioavailability, as measured by the percent of unchanged sulfamethizole excreted following administration of the two sulfamethizole formulations, were not statistically significant at the $p = 0.05$ level. Differences in bioavailability rate parameters were also not significant for the peak excretion times and peak excretion rates of free sulfamethizole.

These results substantiate the view that the absorption of the relatively water soluble sulfamethizole is not dissolution-

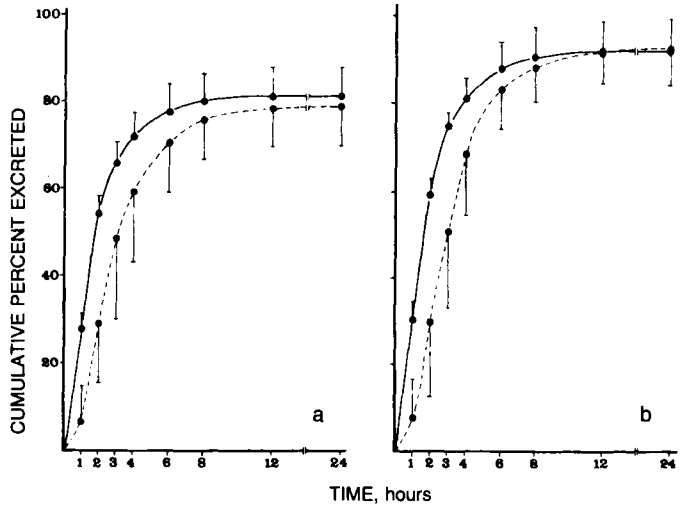

Figure 5—*Mean cumulative percent of unchanged sulfamethizole (a) and "total" sulfamethizole (b) excreted following the administration of formulations A or B. Each data point is the mean ± SD of the cumulative percent excreted for all four subjects. Key: (——) formulation A; (– – –) formulation B.*

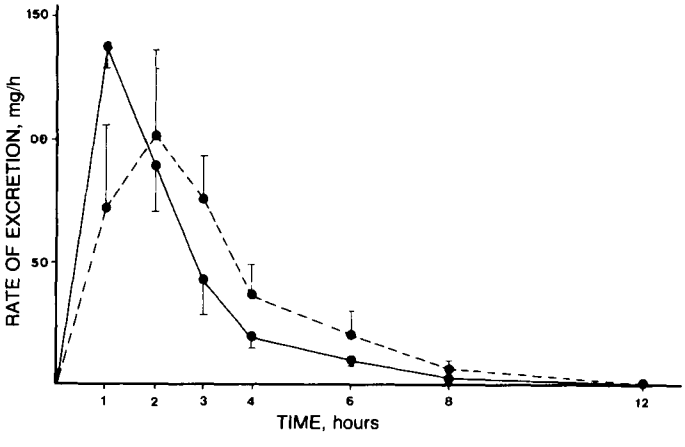

Figure 6—*Mean excretion profiles of free sulfamethizole. Key: (——) formulation A; (– – –) formulation B.*

rate limited and, therefore, the two formulations are bioequivalent in terms of the amount of sulfamethizole excreted.

The results of this study demonstrate the usefulness of the proposed formulations as drug delivery systems of the sparingly water soluble dicumarol and the relatively water soluble sulfamethizole. General conclusions which can be drawn from the present study, however, require consideration of each drug separately.

Previous studies have documented the dissolution rate limited character of dicumarol and, thereby, the formulation sensitivity.[37,41] The present study shows that the problematic dissolution characteristics of dicumarol can be bypassed by the preparation of the freeze-dried product with milk. Unlike other dicumarol formulations, this offers the following advantages: (*a*) consistent absorption; (*b*) because the product is dissolved before ingestion, dissolution studies as part of a bioavailability profile are not necessary; however, in vivo bioequivalence studies will always remain necessary; (*c*) ingesting milk is a daily ritual and eliminates the need for considering food related factors (e.g., type of food, time of interval between eating and drug administration, dietary components). The data for sulfamethizole indicate that the sulfamethizole-milk formulations is bioequivalent to the control capsule with respect to the amount excreted. Therefore, the proposed formulation does not offer any serious advantages over the conventional ones for freely soluble drugs.

In view of all the above, the following general concluding comments can be made: (*a*) the freeze-dried drug–milk formulations are promising as drug delivery systems of sparingly soluble drugs; if, indeed, this is a valid consideration, then definite conclusions should be based on the bioavailability data of the drug under examination; (*b*) further investigations on content uniformity, stability, excipients improving taste, volume, and type of milk, need to be performed to elucidate this novel and attractive formulation technique; (*c*) literature data concerning lipid solubility, protein binding, dissolution and absorption of drugs in presence of milk components,[38,39] and distribution of drugs within milk phases,[42] can be useful for future development of this novel dosage form.

Investigations are currently being conducted to evaluate the importance of these factors and their influence on the bioavailability of drugs.

References and Notes

1. Fincher, J. H.; Adams, J. G.; Beal, H. M. *J. Pharm. Sci.* **1965**, *54*, 704.
2. Atkinson, R. M.; Bedford, C. B.; Child, K. J.; Tomich, E. J. *Nature (London)* **1962**, *193*, 588.
3. Goldberg, A. H.; Gibaldi, M.; Kanig, J. L. *J. Pharm. Sci.* **1965**, *54*, 1145.
4. Goldberg, A. H.; Gibaldi, M.; Kanig, J. L. *J. Pharm. Sci.* **1966**, *55*, 482.
5. Chiou, W. L.; Riegelman, S. *J. Pharm. Sci.* **1971**, *60*, 1281.
6. Chiou, W. L.; Riegelman, S. *J. Pharm. Sci.* **1968**, *58*, 1505.
7. Chiou, W. L. *J. Pharm. Sci.* **1977**, *66*, 989.
8. Stupak, E. I.; Rosenberg, H. A.; Bates, T. R. *J. Pharmacokinet. Biopharm.* **1974**, *2*, 511.
9. Mayersohn, M.; Gibaldi, M. *J. Pharm. Sci.* **1966**, *55*, 1323.
10. Simonelli, A. P.; Mehta, S. C.; Higuchi, W. I. *J. Pharm. Sci.* **1969**, *58*, 538.
11. Stupak, E. I.; Bates, T. R. *J. Pharm. Sci.* **1972**, *61*, 400.
12. Ravis, W. R.; Chen, C. *J. Pharm. Sci.* **1981**, *70*, 1353.
13. Geneidi, A. S.; Al, A. A.; Salama, R. B. *J. Pharm. Sci.* **1978**, *67*, 114.
14. Stavchansky, S.; Gowan, W. G. *J. Pharm. Sci.* **1984**, *73*, 733.
15. Welling, P. G.; Tse, F. L. S. *J. Antimicrob. Chemother.* **1982**, *9*, 7.
16. George, C. I. *Br. Med. J.* **1984**, *289*, 1093.
17. Melander, A. *Clin. Pharmacokinet.* **1978**, *3*, 337.
18. Toothaker, R. D.; Welling, P. G. *Annu. Rev. Pharmacol. Tox.* **1980**, *20*, 173.
19. Welling, P. G. in "Pharmacokinetic Basis for Drug Treatment", Benet, L.; Massoud, N.; Gambertoglio, J., Eds.; Raven Press: New York, 1984; p. 29.
20. Welling, P. G.; Huang, H.; Hewitt, P. F.; Lyons, L. L. *J. Pharm. Sci.* **1978**, *67*, 764.
21. Welling, P. G. *J. Pharmacokinet. Biopharm.* **1977**, *5*, 291.
22. Rasmussen, F. in "Dietary Lipids and Postnatal Development"; Galli, G.; Jacini, G.; Pecile, A., Eds.; Raven Press: New York, 1973; p. 231.
23. Melander, A.; Wahlin, E. *Eur. J. Clin. Pharmacol.* **1978**, *14*, 441.
24. Mattok, G. L.; McGilveray, I. J. *J. Pharm. Sci.* **1972**, *61*, 746.
25. Cho, M. J.; Mitchell, A. G.; Pernarowski, M. *J. Pharm. Sci.* **1971**, *60*, 720.
26. "The Merck Index"; 10th ed.; Windholz, M., Ed.; Merck and Co.: Rahway, NJ, 1983; p. 1278.
27. Nagashima, R.; Levy, G.; Nelson, E. *J. Pharm. Sci.* **1968**, *57*, 58.
28. Bratton, A. C.; Marshall, Jr., E. K. *J. Biol. Chem.* **1939**, *128*, 537.
29. Wagner, J. G. "Biopharmaceutics and Relevant Pharmacokinetics", 1st ed.; Drug Intelligence: Hamilton, IL, 1971; p. 282.
30. Lingnert, H. *J. Food Process. Preserv.* **1980**, *4*, 219.
31. McKenzie, H. A. in "Advances in Protein Chemistry", vol. 22; Anfinsen, C. B., Jr.; Edsall, J. T.; Anson, M. L.; Richards, F. M. Eds.; Academic Press: New York, 1967; p 56.
32. Macheras, P.; Reppas, C., unpublished results.
33. Freese, E.; Levin, B. C.; Pearce, R.; Sreevalsan, T.; Kaufman, J. J.; et al. *Teratology* **1979**, *20*, 413.
34. Koizumi, T.; Arita, T.; Kakemi, K. *Chem. Pharm. Bull. (Tokyo)* **1964**, *12*, 413.
35. Nelson, E. *J. Med. Pharm. Chem.* **1962**, *5*, 211.
36. Morishita, T.; Yamazaki, M.; Yata, N.; Kamada, A. *Chem. Pharm. Bull. (Tokyo)* **1973**, *21*, 2309.
37. Akers, M. J.; Lach, J. L.; Fischer, L. J. *J. Pharm. Sci.* **1973**, *62*, 1192.
38. Rosen, A.; Macheras, P. *J. Pharm. Pharmacol.* **1984**, *36*, 723.
39. Rosen, A.; Macheras, P. *J. Pharm. Pharmacol.* **1985**, *37*, 154.
40. Nagashima, R.; Levy, G.; O'Reilly, R. A. *J. Pharm. Sci.* **1968**, *57*, 1888.
41. Lozinski, E. *Can. Med. Assn. J.* **1960**, *83*, 177.
42. Syversen, G. B.; Ratkje, S. K. *J. Pharm. Sci.* **1985**, *74*, 1071.

Acknowledgments

The authors thank Dr. L. Poulos, Department of Medicine, University of Athens, Athens, Greece, for supervising the in vivo studies.