Bioavailability of 14 Nitrofurantoin Products

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Abstract □ Single lots of 14 commercially available 50- and 100-mg nitrofurantoin products were evaluated *in vitro* and *in vivo*. All products tested met USP XVIII specifications for drug content, disintegration time, and dissolution rate. Statistically significant differences were observed in bioavailability, as determined from crossover urinary excretion studies in 14 human volunteers. The cumulative amount of drug excreted and the duration of therapeutic urine levels were compared. No useful correlation was observed between the extent of urinary excretion and either the disintegration or dissolution characteristics of the dosage forms tested.

Keyphrases □ Nitrofurantoin—bioavailability of 14 commercially available 50- and 100-mg products, attempted *in vivo–in vitro* correlations □ Bioavailability—14 commercially available 50- and 100-mg products, correlations between urinary excretion and disintegration/dissolution characteristics attempted

Numerous recent reports (1–4) provided evidence to support the contention that not all commercially available products containing a given drug will necessarily exhibit equivalent bioavailability. While compendial assay procedures may indicate that each product has essentially identical content, the rate and extent of absorption of the drug from the dosage form following oral administration may differ significantly from product to product.

The urinary tract, antibacterial drug nitrofurantoin is one therapeutic agent that has exhibited variable bioavailability between chemically equivalent products (5–8). In recognition of a potential for bioavailability problems, nitrofurantoin was considered in the recent APhA Bioavailability Pilot Project (9).

The present report deals with a crossover comparison of the bioavailability of 14 nitrofurantoin products. Urinary excretion studies were employed since this drug is excreted in the urine approximately 40% as unchanged drug following oral administration in humans (5–8), and the urinary tract is the site of therapeutic activity. The drug has a half-life in humans of less than 1 hr (5), and clearance is not dependent on urinary pH (8).

EXPERIMENTAL

Analysis of Urine Samples—A method nearly identical to that of Conklin and Hollifield (10) was employed in the determination of nitrofurantoin excreted in the urine. The method involved acidification of 0.5 ml of urine with 2.0 ml of 0.2 *N* HCl and extraction with 5.0 ml of nitromethane. Three milliliters of the nitromethane layer was combined with 0.5 ml of 0.04 *M* hyamine hydroxide in absolute methanol. The absorbance of the resulting solution was determined at 400 nm against a blank sample prepared by carrying 0.5 ml of distilled water through the extraction procedure. Blank urine samples, collected prior to drug administration, were also analyzed; each drug absorbance reading was corrected for the blank urine value.

Product Composite Assay—Twenty tablets or the contents of 20 capsules of each product were finely powdered and six aliquots, each equivalent to approximately 50 mg of nitrofurantoin, were accurately weighed. Approximately 10 ml of dimethylformamide (I) was added to each portion. Following thorough mixing, the samples were filtered through a glass-fiber filter, with adequate rinsing to ensure quantitative transfer. The resulting filtrate was diluted to 50 ml with I, and 1 ml of this solution was diluted to 25 ml with a 10% aqueous solution of I. One-milliliter samples of these solutions were then carried through the assay extraction procedure employed for the urine samples. Blank values were derived from the extraction of 1.0 ml of a 10% aqueous solution of I.

Content Uniformity—The contents of 10 individual capsules or 10 tablets of each 50- or 100-mg product were crushed into fine powders, and each individual powder was assayed as described in the composite assay, except that the 100-mg nitrofurantoin samples were diluted to 50 ml, prior to extraction, with a 10% solution of I.

USP Disintegration Test—Six dosage units of all 14 products were subjected to the USP XVIII disintegration test. The tests were carried out in distilled water at 37° with disks, using a USP tablet disintegration tester[1].

Dissolution Test—Six dosage units of all 14 products were subjected to the USP XVIII dissolution test. The determinations were made in a six-chamber dissolution apparatus[2] at 37°. Each dosage unit was placed into a wire-mesh dissolution basket and submersed in 900 ml of pH 7.2 phosphate buffer. The basket was rotated at 100 rpm; 5-ml samples were withdrawn at 5, 10, 15, 20, 30, 40, 50, 60, 75, and 90 min after the basket was introduced into the buffer solution. The withdrawn samples were assayed for nitrofurantoin content by diluting 1.0 ml of each sample to 10.0 ml with buffer and determining the absorbance at 380 nm, using a pH 7.2 phosphate buffer as the blank.

Clinical Study Protocol—The average age of the subjects[3] was 24 years (range 21–32), the average weight was 78.7 kg (range 61.2–106.6), and the average height was 175.6 cm (range 167.6–190.5). Each volunteer was screened regarding general health and any known drug allergies. In addition, all subjects underwent a hematologic and SMA 12/60 blood analysis to ensure inclusion of only those subjects in good health. Each subject gave written informed consent to participate in the study.

All subjects were instructed to refrain from taking any medication during the study. Each subject was given a single dose of a nitrofurantoin product once a week for 14 weeks. The dose was taken in the morning, following an overnight fast. No food or liquid, other than water, was permitted until 4 hr following ingestion of the dose. A blank urine sample was voided prior to taking the dose, and urine samples were collected at 1, 1.5, 2, 3, 4, 6, and 12 hr postadministration.

The total volume of the sample was recorded, along with the time of collection, and at least a 10-ml portion of the sample was placed into a small glass vial with a plastic snap-cap. The samples were frozen until the day of analysis. At the time of assay, the sample was thawed and analyzed for nitrofurantoin concentration in the previously described manner.

The sequence in which each subject received each drug each week is summarized in Table I. The sequence follows essentially the pattern suggested by Williams (11) and is designed to minimize the influence of any cumulative or residual effects of preceding doses. Due to technical difficulties, during the 1st week of the study the precise design (11) was not employed. Each subject repeated his 1st week drug following his 14th week drug, and the results of the 1st week were discarded. Furthermore, Subject 14 was dosed partially out of sequence. However, residual or cumulative effects were not anticipated due to the short half-life of the drug and the 1-week interval between doses.

[1] SGA Scientific Inc., Bloomfield, N.J.
[2] Hansen Research Corp., Northridge, Calif.
[3] Fourteen paid male volunteers were recruited from the student body of the University of Tennessee College of Pharmacy.

Table I—Experimental Design for Nitrofurantoin Bioavailability Evaluation[a]

Subject	Week													
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
1	2	14	3	13	4	12	5	11	6	10	7	9	8	1
2	3	1	4	14	5	13	6	12	7	11	8	10	9	2
3	4	2	5	1	6	14	7	13	8	12	9	11	10	3
4	5	3	6	2	7	1	8	14	9	13	10	12	11	5
5	6	4	7	3	8	2	9	1	10	14	11	13	12	5
6	7	5	8	4	9	3	10	2	11	1	12	14	13	6
7	8	6	9	5	10	4	11	3	12	2	13	1	14	7
8	9	7	10	6	11	5	12	4	13	3	14	2	1	8
9	10	8	11	7	12	6	13	5	14	4	1	3	2	9
10	11	9	12	8	13	7	14	6	1	5	2	4	3	10
11	12	10	13	9	14	8	1	7	2	6	3	5	4	11
12	13	11	14	10	1	9	2	8	3	7	4	6	5	12
13	14	12	1	11	2	10	3	9	4	8	5	7	6	13
14	1	13	12	3	11	2	4	10	5	9	6	8	7	14

[a] Each number within the matrix corresponds to a specific product lot: No. 1 was 50-mg tablets from Ketchum Laboratories (Lot 204145); No. 2 was 50-mg tablets from Lederle Laboratories (Lot 286-108); No. 3 was 100-mg tablets from Ketchum Laboratories (Lot 2011460); No. 4 was 100-mg tablets from McKesson Laboratories (Lot 2B039); No. 5 was 100-mg tablets from Purepac Pharmaceutical Co. (Lot 1061460); No. 6 was 100-mg tablets from Eaton Laboratories (Lot 698627); No. 7 was 100-mg macrocrystal capsules from Eaton Laboratories (Lot 697203); No. 8 was 50-mg tablets from Purepac Pharmaceutical Co. (Lot 090009); No. 9 was 50-mg tablets from Eaton Laboratories (Lot 693775); No. 10 was 50-mg macrocrystal capsules from Eaton Laboratories (Lot 698613); No. 11 was 50-mg tablets from McKesson Laboratories (Lot 2J784); No. 12 was 100-mg tablets from Lederle Laboratories (Lot 286-104); No. 13 was 100-mg Zambon tablets from Wolins Pharmacal (Lot 24015); and No. 14 was 50-mg Zambon tablets from Wolins Pharmacal (Lot 24732).

Selection of Nitrofurantoin Products—Table I also summarizes the nitrofurantoin products evaluated. Six manufacturers or distributors are represented, with a single lot of at least two product strengths tested for each. In each instance the products tested were purchased directly from a local pharmacy or drug wholesaler to ensure that the products were representative of those currently available for dispensing.

RESULTS AND DISCUSSION

Product Composite Assay—The results of the composite analyses of the 14 product lots are summarized in Table II, expressed in terms of the percent of labeled content actually found by analysis. Each of the 14 products was within the prescribed USP XVIII limits of 95.0–105.0% of the labeled content.

Content Uniformity Analysis—The USP XVIII requires that the assay of 10 individual tablets of each lot should indicate that not less than nine of the tablets are within 85–115% of the average of the tolerances specified in the potency definition in the monograph and that the content of none of the tablets falls outside the limits of 75–125% of that average. Table III summarizes the results of the content uniformity determination of 10 tablets or capsules of each of the 14 nitrofurantoin product lots. In no instance did any of the products fail to meet the USP XVIII specifications.

Disintegration Testing—The USP XVIII requires the testing of six tablets of each product lot for disintegration properties and specifies that none of the six tablets should fail to disintegrate in less than 30 min. If one or two tablets fail the test, an additional 12 tablets are to be tested and not less than 16 of the tablets must disintegrate within 30 min. As may be seen from Table IV, all 14 products tested disintegrated well within the 30-min time limit, with the mean times ranging from 1.3 to 16.4 min.

Dissolution Rate Testing—The USP XVIII nitrofurantoin monograph specifies that the time required for 60% of the labeled amount to dissolve is not less than 60 min. This is in contrast to USP XVIII monographs for other drugs, which require that a certain percent of the dose must dissolve within a specified period. Inconsistencies in the USP XVIII dissolution rate specifications for nitrofurantoin were recently discussed (12). For nitrofurantoin, if one or two dosage units fail to meet the requirements, the test is repeated with six additional dosage units, and not less than 10 of the 12 units tested must pass.

Table V summarizes the dissolution data obtained for each of the 14 products. With the exception of Products 5 and 8, all products were significantly less than 50% dissolved at the 60-min sampling time and, therefore, met the USP specifications. Because two of the six tablets tested for Product 8 were more than 60% dissolved after 60 min, and five tablets of Product 5 were close to 60% dissolved after this time, an additional six tablets of each of these products were tested. Of the 12 total tablets tested for Products 5 and 8, three and four tablets, respectively, dissolved more rapidly than permitted by the USP XVIII specifications.

Strictly speaking, these two products failed to meet the USP requirements for dissolution, but the clinical significance of this specification is debatable. The apparent intent of the USP dissolution test is to provide for a relatively slowly dissolving dosage form,

Table II—Twenty Tablet USP Composite Assay for Nitrofurantoin Products

Product[a]	Dose, mg	Mean Percent of Labeled Content[b]	*SD*
9	50	105.43	1.64
14	50	105.15	1.78
10	50	104.67	2.30
12	100	102.92	1.41
11	50	102.89	1.15
6	100	102.83	1.15
8	50	102.60	3.88
4	100	102.41	1.79
5	100	101.91	2.71
1	50	101.50	1.91
13	100	101.08	2.74
7	100	100.06	2.09
3	100	99.40	1.66
2	50	96.12	3.50

[a] See Table I for product code numbers. [b] Mean of six determinations.

Table III—USP XVIII Content Uniformity Analysis of Nitrofurantoin Products

Product[a]	Dose, mg	Mean Percent of Labeled Content[b]	*SD*
9	50	107.07	2.09
7	100	106.71	4.63
14	50	106.41	4.01
10	50	106.01	6.40
5	100	104.70	3.74
8	50	103.99	4.21
12	100	103.55	2.99
4	100	103.54	6.30
6	100	103.26	3.38
11	50	102.56	3.71
13	100	101.06	3.26
3	100	100.96	6.76
1	50	100.84	3.84
2	50	98.12	3.42

[a] See Table I for product code numbers. [b] Mean of six determinations.

Table IV—USP XVIII Disintegration Test for Nitrofurantoin Products

Product[a]	Dose, mg	Mean Disintegration Time[b], min	*SD*
8	50	1.30	0.26
5	100	8.26	3.43
9	50	8.96	1.40
10	50	8.98	0.62
2	50	9.97	2.57
14	50	10.20	1.56
6	100	11.26	3.70
7	100	11.27	0.65
12	100	12.10	1.93
4	100	12.30	1.58
11	50	13.38	3.23
13	100	13.64	1.09
3	100	15.94	0.59
1	50	16.42	1.36

[a] See Table I for product code number. [b] Mean of six determinations.

Table VI—One-Way Analysis of Variance of Percent Dissolution of Nitrofurantoin Products after 90 min[a]

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	*F* Ratio
Between products	13	38.84	2.99	66.98
Within products	70	3.12	0.04	
Total	83	41.97		

Rank Order of Products in Terms of Mean Percent Dissolved after 90 min[b] (Least to Most Dissolved Product)[c]

(4) 14 (13) 11 (3) 2 9 1 (12) (6) 10 (7) (5) 8

[a] Using Cochran's test for homogeneity of variance. All data were subjected to a ln (X) transformation because of a significant nonadditivity in the untransformed data. [b] Products ranked on the basis of the Newman–Keuls *a posteriori* test. Products underlined by the same line are not significantly different ($p > 0.01$). [c] See Table I for product code numbers; 100-mg products are indicated by parentheses.

which presumably will be less prone to elicit GI distress in susceptible individuals. In the present study, 196 doses of nitrofurantoin were administered and only one incident of nausea and vomiting was reported.

Table VI summarizes the statistical analysis of the dissolution data, comparing the mean percent dissolved from each of the 14 products after 90 min. Because of a significant nonadditivity in the dissolution data, the analysis was conducted on natural log transformed data which reduced but did not eliminate the nonadditivity. The analysis indicates that the dissolution data may be approximately divided into three dissolution groups, with Products 8, 5, 7, 10, and 6 being relatively rapidly dissolved, Products 4, 14, 13, 11, and 3 being relatively slowly dissolved, and the remainder having an intermediate dissolution rate.

Urinary Excretion Studies—Figure 1 summarizes the average data from the urinary excretion studies of all 14 products in the 14 subjects. The mean cumulative percent of nitrofurantoin excreted for Products 14 and 11 was less than half that observed following administration of Product 9. A two-way analysis of variance of the urinary excretion data was conducted for each urine sampling time. At the early sampling times, 1 through 3 hr postadministration, the data exhibited considerable variability between subjects for each product, as indicated by a relatively large standard deviation and relative standard deviation. As the sampling continued through 12 hr, the intersubject variability continually declined. From 3 hr on, Products 14, 11, 10, and 7 began to emerge as being less bioavailable than the other products being evaluated. Table VII summarizes the two-way analysis of variance for products at the 12-hr sampling time.

The week's effect (in conjunction with the product and subject effects) was examined using a general linear regression model and, as expected, was not significant for any analysis ($p = 0.05$). Therefore, the week's sums of squares, with its accompanying degrees of freedom, was added into the error terms. Products 14 and 11 were significantly ($p < 0.01$) less bioavailable than the other products. While Products 10 and 7 were the next least bioavailable, the differences were not statistically significant compared to the other products tested.

There was no apparent relationship between the dose (50 or 100 mg) and the percent of drug excreted. The two products exhibiting the poorest bioavailability were 50-mg tablets. However, studies of the 100-mg tablets obtained from the same sources as the 50-mg tablets indicated satisfactory bioavailability. In the absence of knowledge of the components of the formulations and the manufacturing process, it is not possible to explain the observed differences in bioavailability.

In addition to examining the cumulative percent of nitrofurantoin excreted following administration of each product, the urine levels of nitrofurantoin were compared. Previous reports indicated that a urine concentration of about 30 μg/ml should be effective against 90% of the strains of *Escherichia coli*, although levels of 75 μg/ml may be required against some resistant strains (13). Urine nitrofurantoin concentrations at each sampling time, for each product, in each subject, were plotted *versus* the time each sample was collected. The graphical points were connected by straight lines, and the duration of time for the maintenance of the 30- and 75-μg/ml levels was measured from each graph. The procedure, while somewhat imprecise due to unequal sampling intervals, still afforded the opportunity to identify further two products that

Table V—USP XVIII Dissolution Test of Nitrofurantoin Products

Product[a]	Dose, mg	Mean Percent Dissolved[b] 15 min	30 min	60 min	90 min
8	50	28.66 (1.65)	41.98 (3.45)	57.58 (3.59)	69.71 (4.31)
5R	100	21.97 (8.53)	39.53 (8.63)	57.43 (9.14)	66.90 (8.05)
5	100	19.99 (6.23)	37.72 (4.96)	55.11 (6.25)	65.12 (3.98)
8R	50	25.36 (4.69	38.03 (6.90)	52.20 (9.11)	62.03 (9.62)
7	100	13.03 (2.12)	24.81 (4.29)	39.66 (7.05)	50.26 (8.93)
10	50	12.61 (1.93)	22.77 (2.46)	38.31 (4.12)	47.46 (4.79)
6	100	9.66 (1.42)	19.71 (2.88)	34.56 (2.74)	45.18 (3.68)
1	50	1.80 (0.51)	7.39 (3.54)	28.19 (10.83)	38.34 (14.75)
12	100	3.66 (1.44)	16.08 (3.96)	29.49 (4.90)	36.94 (4.75)
9	50	3.86 (1.23)	9.21 (2.48)	17.86 (5.49)	24.82 (6.64)
2	50	1.98 (2.29)	8.21 (4.81)	18.37 (6.83)	24.23 (9.13)
3	100	1.01 (0.34)	3.54 (1.30)	11.13 (2.77)	16.66 (3.05)
11	50	3.42 (0.82)	5.78 (1.58)	9.99 (1.55)	12.75 (1.92)
13	100	3.34 (0.39)	5.17 (0.93)	9.70 (2.25)	12.47 (2.87)
4	100	2.42 (1.13)	4.67 (2.06)	7.52 (1.96)	9.67 (2.42)
14	50	3.21 (0.93)	4.60 (0.56)	7.60 (0.68)	9.54 (1.05)

[a] See Table I for product code numbers; 5R and 8R represent repeat determinations. [b] Mean of six determinations. Data are expressed in terms of percent of labeled content, with standard deviations in parentheses.

Table VII—Two-Way Analysis of Variance of Cumulative Percent of Nitrofurantoin Excreted 12 hr Postadministration Using Tukey's Test for Nonadditivity[a]

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	*F* Ratio
Subjects	5.84	13	0.45	5.22
Products	18.45	13	1.42	16.50
Nonadditivity	0.22	1	0.22	2.50
Balance	14.46	168	0.09	
Total	38.96	195		

Rank Order of Products 12 hr Postadministration[b] (Least to Most Bioavailable Product)[c]

14 11 10 (7) (3) (12) 2 (4) (6) (13) 1 8 (5) 9

[a] All data were subjected to a ln $(X + 0.01)$ transformation because of a significant nonadditivity in the untransformed data. [b] Products ranked on the basis of the Newman–Keuls *a posteriori* test. Products underlined by the same line are not significantly different ($p > 0.01$). [c] See Table I for product code numbers; 100-mg products are indicated by parentheses.

Table VIII—Duration (Hours) for Maintenance of Urine Nitrofurantoin Levels of 30 or 75 μg/ml

Product[a]	Dose, mg	Duration, hr[b] 30 μg/ml	75 μg/ml
6	100	5.50 (1.63)	2.32 (1.58)
4	100	5.27 (2.07)	1.82 (1.89)
3	100	4.93 (2.06)	2.48 (1.66)
12	100	4.87 (2.14)	2.20 (1.66)
5	100	4.79 (2.38)	2.60 (2.13)
13	100	4.77 (2.29)	2.23 (2.05)
7	100	4.69 (1.68)	1.91 (1.55)
9	50	3.88 (1.98)	1.30 (1.47)
1	50	3.30 (2.27)	1.34 (1.33)
8	50	3.11 (1.37)	1.56 (1.30)
10	50	2.59 (2.18)	0.50 (0.99)
2	50	2.38 (1.92)	0.99 (1.41)
14	50	0.51 (0.98)	0.11 (0.41)
11	50	0.49 (0.81)	0.10 (0.26)

[a] See Table I for product code numbers. [b] Mean value for 14 subjects, with standard deviation in parentheses.

provided relatively low urine drug levels. Table VIII summarizes the mean duration that each drug product maintained the specified urine levels, and Tables IX and X give the results of the two-way analysis of variance of the duration data. Products 14 and 11 provided a significantly ($p < 0.01$) shorter duration of the 30-μg/ml drug level, with no statistically significant difference between the other products tested. Products 14 and 11 also provided a shorter duration of the 75-μg/ml drug level ($p < 0.01$).

Differences observed between the various other products are summarized in Tables IX and X. While Product 7 was ranked as being relatively poor in terms of cumulative percent excreted, it provided for a duration of urine drug levels comparable to the other products tested. Even though this product was less completely available for absorption, the rate and extent of absorption were apparently sufficient to provide adequate urine drug levels. The 100-mg products provided the highest levels, as would be expected if the assumed direct relationship between dose absorbed and quantity of drug excreted was valid.

It appears that the relatively poor bioavailability of Products 14 and 11, as determined from total cumulative percent of drug excreted and the relatively short duration of urine levels recognized as clinically effective, would strongly suggest that these two products may not compare favorably with the other products tested in terms of therapeutic equivalency.

***In Vitro–In Vivo* Correlations**—Attempts were made to correlate the mean *in vitro* disintegration time of samples of each of the 14 nitrofurantoin products with the mean cumulative percent of nitrofurantoin excreted in the urine after 12 hr. While Fig. 2 indicates the expected trend of a decreasing cumulative percent excreted with increasing disintegration time, the correlation ($r = -0.23$) is not sufficient to provide for a reliable prediction of nitrofurantoin bioavailability from the disintegration test. The correlation of mean disintegration time with cumulative percent excreted after 1 and 2 hr was slightly improved, $r = -0.32$ and $r = -0.36$, respectively, but was insufficient for accurate prediction of *in vivo* availability.

Correlations between bioavailability and dissolution rate of solid dosage forms have been reported to be more meaningful than correlations with disintegration time for certain drugs (14, 15). Figure

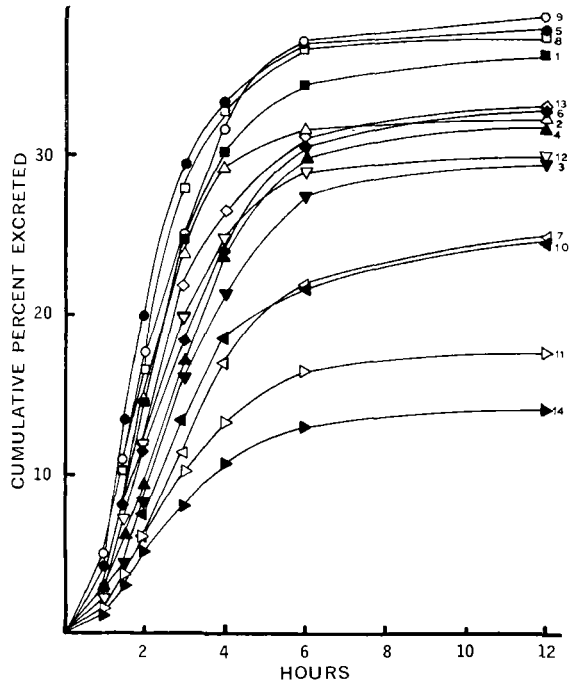

Figure 1—*Mean cumulative percent of nitrofurantoin excreted following the oral administration of 14 nitrofurantoin products. Product code numbers (Table I) appear beside the 12-hr sample. Each data point is the mean cumulative percent excreted for all 14 subjects.*

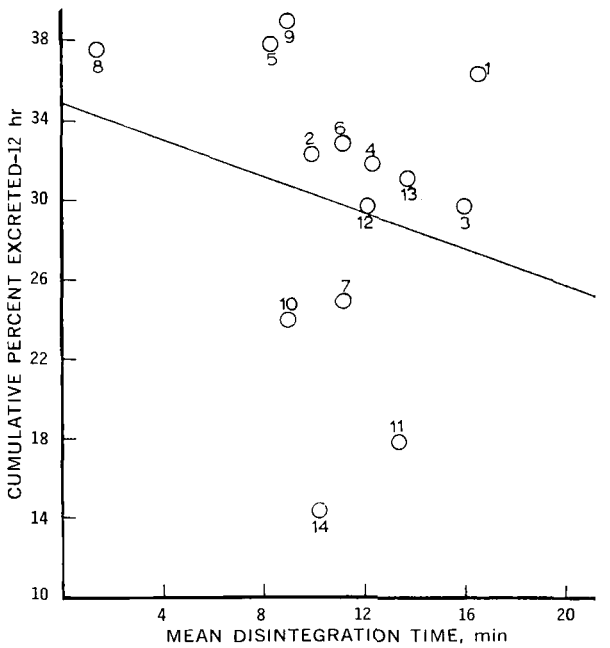

Figure 2—*Correlation of mean disintegration time with cumulative percent of nitrofurantoin excreted after 12 hr. Cumulative percent excreted values are mean values from 14 subjects; disintegration times are means of six determinations. Product code numbers (Table I) appear beside each data point.*

Table IX—Two-Way Analysis of Variance for Duration of 30-μg/ml Urine Levels[a]

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	*F* Ratio
Subjects	17.82	13	1.37	0.55
Products	447.68	13	34.44	13.80
Nonadditivity	0.93	1	0.93	0.37
Balance	419.17	168	2.50	
Total	885.59	195		

Rank Order of Products[b] (Least to Greatest Duration of 30-μg/ml Urine Level by Product)[c]

14 11 10 2 1 8 (12) 9 (5) (13) (7) (3) (4) (6)

[a] All data were subjected to a ln $(X + 0.01)$ transformation because of a significant nonadditivity, using Tukey's test, in the untransformed data. [b] Products ranked on the basis of the Newman–Keuls *a posteriori* test. Products underlined by the same line are not significantly different ($p > 0.01$). [c] See Table I for product code numbers; 100-mg products are indicated by parentheses.

Table X—Two-Way Analysis of Variance for Duration of 75-μg/ml Urine Levels[a]

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	*F* Ratio
Subjects	32.97	13	2.54	0.50
Products	406.19	13	31.25	6.11
Nonadditivity	22.54	1	22.54	4.41
Balance	858.64	168	5.11	
Total	1320.34	195		

Rank Order of Products[b] (Least to Greatest Duration of 75-μg/ml Urine Levels by Product)[c]

14 11 10 2 9 1 (4) 8 (5) (7) (13) (12) (6) (3)

[a] All data were subjected to a ln $(X + 0.01)$ transformation because of a significant nonadditivity, using Tukey's test, in the untransformed data. [b] Products ranked on the basis of the Newman–Keuls *a posteriori* test. Products underlined by the same line are not significantly different ($p > 0.01$). [c] See Table I for product code numbers; 100-mg products are indicated by parentheses.

3, however, shows the relatively poor correlation ($r = 0.45$) for mean percent of nitrofurantoin dissolved after 90 min *versus* cumulative percent excreted after 12 hr. As was the case with the attempted correlation with disintegration time, the *in vitro* test was not a reliable predictor of *in vivo* availability. Even poorer results were obtain for attempted correlations between cumulative percent excreted after 12 hr and mean percent dissolved after 15 min ($r = 0.29$), 30 min ($r = 0.36$), and 60 min ($r = 0.43$). Thus, while the attempted correlations indicated that the quantity of nitrofurantoin absorbed increased with an increasing dissolution rate, the prediction of *in vivo* bioavailability was not sufficiently precise to permit application of the *in vitro* testing procedure to evaluate the products.

SUMMARY AND CONCLUSIONS

The results of the evaluation of single lots of 14 different nitrofurantoin products indicated that all products tested exhibited chemical and physical properties that were within acceptable limits, as specified by USP XVIII. Urinary excretion determinations revealed that two lots were significantly less bioavailable than the others tested. Two products available in the form of macrocrystals were less bioavailable than the majority of the products tested, but urine drug levels appeared adequate, and differences between these products and the 10 most readily absorbed products were not statistically significant. No adequate correlation was observed between the extent of *in vivo* bioavailability and either the *in vitro* disintegration time or dissolution rate.

While the present investigation does not consider the issue of whether other lots of a nitrofurantoin product from a given company will necessarily be identical to the lots tested, the results do indicate that certain companies have marketed products that satisfy present USP specifications but exhibit unsatisfactory bioavailability.

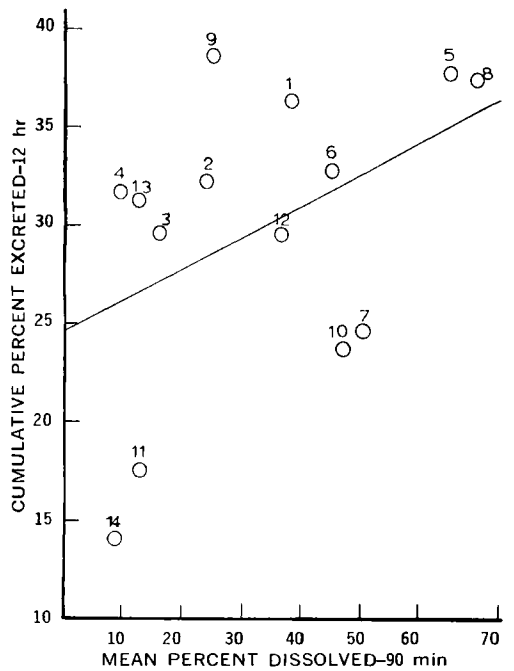

Figure 3—*Correlation of mean percent dissolved after 90 min with cumulative percent excreted after 12 hr. Cumulative percent excreted values are mean values from 14 subjects; dissolution values are means of six determinations. Product code numbers (Table I) appear beside each data point.*

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 28, 1974, from the *Division of Drug Metabolism and Biopharmaceutics, Department of Medicinal Chemistry, College of Pharmacy, University of Tennessee Medical Units, Memphis, TN 38163*

Accepted for publication June 21, 1974.

Supported in part by a contract from the Tennessee Department of Public Health and by U.S. Public Health Service Grant HL-09495.

The authors thank Mrs. Irma Miller for her technical assistance, Mrs. Ann McEachran for her assistance in the statistical analysis of the data, Dr. Phillip Lieberman for providing the medical supervision of the study, Mr. Charles Cruze for assisting in the computer analysis of the data, and the 14 subjects who volunteered to participate in the study. Special thanks are extended to Mr. Herbert Bates for his encouragement and support.

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Critical Evaluation of Use of Effective Protein Fractions in Developing Pharmacokinetic Models for Drug Distribution

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Abstract □ A critical evaluation of the concept of effective protein fractions, which was previously utilized in physiologically based pharmacokinetic models to describe the binding of barbiturates to plasma and various tissues, is presented. The calculation of effective protein fractions requires as a minimum the extrapolation of *in vitro* binding parameters from one concentration of protein to another. Based on available literature data, it is shown that thiopental binding parameters vary markedly with the concentration of bovine serum albumin and that such concentration dependency cannot be predicted readily. Similar anomalous protein binding behavior has been reported for other drugs. Consequently, the use of effective protein fractions in developing pharmacokinetic models for drug distribution may not generally be feasible. The apparent successful use of such fractions in the case of thiopental appears to be fortuitous. Errors incurred in the extrapolation of the binding parameters may be compensated for by errors introduced by the experimental method in which the original binding data were obtained as well as interspecies differences in protein binding.

Keyphrases □ Protein fractions, effective—evaluation of use in developing drug distribution pharmacokinetic models □ Pharmacokinetic models—effective protein fraction, barbiturate binding, evaluation of use, equations □ Drug distribution—pharmacokinetic models, evaluation of effective protein fraction □ Binding parameters—evaluation of use of effective protein fraction in pharmacokinetic drug distribution models

In recent years the utility of physiologically based pharmacokinetic models has been demonstrated in describing quantitatively the distribution and elimination of barbiturates (1, 2), methotrexate (3–5), and cytarabine (cytosine arabinoside) (6) in various species. These models are uniquely different from the conventional compartmental models because all parameters involved have a specific physiological and/or physicochemical basis.

The development of such models carried the exciting implication that, in principle, based on *in vitro* tissue binding and partition studies together with well-documented physiological parameters such as organ weight and blood flow rate, it may be possible to make *a priori* predictions of drug distribution in the body. Excretion parameters such as biliary and renal clearances can be obtained from appropriate animal data. With the cytarabine model, an initial attempt was made to incorporate *in vitro* enzyme kinetic data in quantitating metabolism. It is evident that the development of physiologically and anatomically realistic models offers the possibility of a systematic, rational approach in extrapolating animal data to humans by appropriate scale-up of parameters.

DISCUSSION

In general, the four-compartment model proposed for barbiturates (1) and, in particular, for thiopental (2) represents the basic framework of all physiological models. The body is divided into several well-defined anatomical regions: blood, viscera, lean, and adipose compartments. Each body region further consists of a blood pool in equilibrium with the respective tissue. Within each compartment, except for the adipose tissue, the drug is present in both freely diffusible and "protein"[1]-bound form. Mathematical resolution of the model, therefore, requires binding parameters relating the free and bound concentrations in the various compartments.

In principle, the binding relationships should be readily obtainable by *in vitro* binding studies with plasma and various tissues. Abundant information on drug–plasma protein interaction already exists in the literature, whereas tissue binding data are meager or nonexistent. Furthermore, tissue proteins are poorly defined. Tissue samples are often too insoluble, and only their homogenates or centrifugal fractions can be studied. But plasma binding studies can easily be performed with whole plasma, plasma protein fractions, or crystalline serum albumin. The latter is often preferred because it is commercially available in pure form, is well characterized, and constitutes about 50–65% of total plasma protein, *i.e.*, 3.5–5.5% by weight of plasma.

Binding data obtained with an isolated protein such as albumin can be analyzed according to the physical law of mass action. Multiple classes of noninteracting binding sites are often assumed, and

[1] The term "protein," as used in this article, refers to those components in various tissues and blood to which the drug is bound. These components include proteins and other macromolecules.