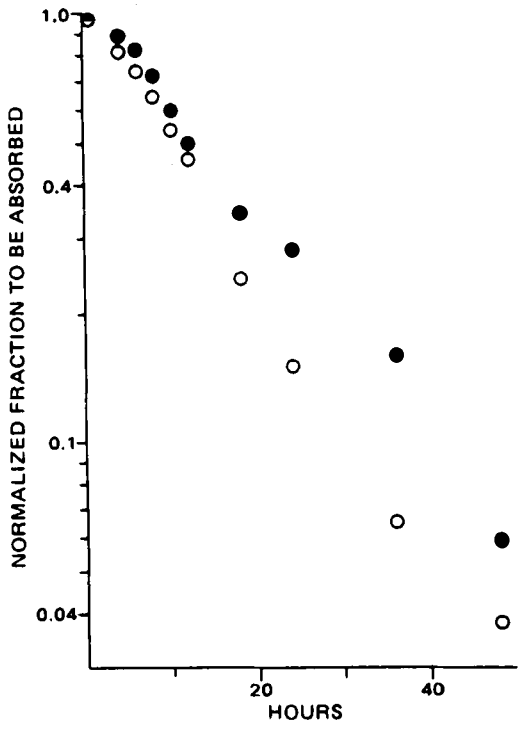

Figure 4—*Dicumarol absorption kinetics in four control (○) and four oral phenobarbital-treated (●) rats, all with ligated bile ducts. Plotted on the ordinate is the fraction of total absorbed dicumarol that was remaining to be absorbed at various times after oral administration of a 50-mg/kg dose.*

Dicumarol was absorbed much more slowly by bile duct-ligated animals ($t_{50\%} \simeq 11$ hr) than by normal rats ($t_{1/2} \simeq 3$ hr; Ref. 2). The reason for this difference is not evident from the available data. Bile duct-ligated control rats absorbed ≤40% of the oral dose while normal control rats absorbed >80% under the same experimental conditions (2). Perhaps bile enhances absorption by increasing the dissolution rate of the almost water-insoluble dicumarol. It will be of interest to determine systemic dicumarol availability in rats with exteriorized bile ducts who are receiving a concomitant intravenous bile infusion, *i.e.*, animals that are not cholestatic or bile salt deficient and presumably have normal liver function but no bile in the intestine.

Systemic dicumarol availability in normal rats was reduced from >80 to <50% by phenobarbital treatment (2). No such absorption inhibitory effect was observed in bile duct-ligated rats. Dicumarol availability is likely to be affected by many factors such as the solubilizing effect of intestinal fluids, GI motility, and gut wall metabolism. (Significant first-pass hepatic biotransformation can be excluded on theoretical grounds; Ref. 6.) Since bile duct ligation changed the pathophysiological status of the animals (rather than only preventing bile entry into the intestine), the lack of a significant phenobarbital effect on systemic dicumarol availability in bile duct-ligated rats cannot be ascribed to any one factor. However, the results of this investigation demonstrate that a drug–drug interaction such as the one between dicumarol and phenobarbital may be pronounced in normal animals and either absent or less pronounced in animals with altered pathophysiological status.

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Double Latin Square Study to Determine Variability and Relative Bioavailability of Methylprednisolone

K. S. ALBERT[x], S. W. BROWN, Jr., K. A. DeSANTE, A. R. DiSANTO, R. D. STEWART, and T. T. CHEN

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Abstract □ The variability and relative bioavailability of methylprednisolone tablets were evaluated utilizing a double Latin square crossover design in which each of 20 subjects was given four of five treatments. Three different lots of methylprednisolone tablets exhibited virtually identical absorption, with similar ranges and coefficients of variation of some selected bioavailability parameters indicative of lot-to-lot uniformity in bioavailability. Within-lot and between-lot uniformities in bioavailability also were similar, suggesting that the observed variability in serum methylprednisolone levels was not due to manufacturing process variables. With respect to intra- *versus* intersubject variability, no differences were found for the absorption rate or terminal half-life. In contrast, between-subject variability associated with extent of absorption was greater than that within subjects. Relative to an aqueous suspension, methylprednisolone tablets were fully bioavailable.

Keyphrases □ Methylprednisolone—bioavailability, tablet variability, commercial preparations, Latin square study □ Glucocorticoids—methylprednisolone, bioavailability, tablet variability, commercial preparations, Latin square study □ Bioavailability—commercial methylprednisolone preparations

On January 7, 1977, the Food and Drug Administration published a list of rules and regulations for conducting bioequivalency studies in humans (1). Their intent was to assure product interchangeability by demonstrating that, on the average, two or more products would exhibit similar bioavailabilities. However, the rules and regulations appear to have ignored the variability in the bioavailability of a given product; and this variability might result in thera-

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Table I—Dosage Schedule

Group	Subjects per Group	Subjects in Group[a]	Treatment Assignments Phase I	Phase II	Phase III	Phase IV
I	2	2, 17	A	B	D	C
II	2	1, 20	B	C	A	D
III	2	4, 5	C	D	B	A
IV	2	8, 19	D	A	C	B
V	3	9, 11, 13	A	B	E	C
VI	3	6, 14, 18	B	C	A	E
VII	3	7, 12, 16	C	E	B	A
VIII	3	3, 10, 15	E	A	C	B

[a] Subjects were randomly assigned to each group.

peutic failure or untoward drug reactions in a given segment of the patient population.

Variability for a product may be documented by evaluating its lot-to-lot uniformity in bioavailability. By administering at least one of the lots on two separate occasions, information on variability within a given lot as well as between lots can be obtained.

This paper describes results of a bioavailability study designed to document the lot-to-lot variability and relative bioavailability of methylprednisolone tablets. With respect to the rate and extent of absorption, within-lot and between-lot uniformities in bioavailability were similar, suggesting that the observed variability in serum methylprednisolone levels was free from manufacturing influences.

EXPERIMENTAL

The 20 normal, nonobese male volunteers, whose average age was 22 years (19–27 years) and whose average weight was 74.8 kg (63–97 kg), exhibited normal vital signs and selected laboratory parameters and were without any evidence of cardiac, renal, or GI abnormalities. Subjects did not receive any repository steroid preparation for 60 days prior to the study or any other steroid or nonsteroid product, including topical preparations, for 14 days before the protocol was initiated. During the study, volunteers received only the medication prescribed, with 5 days separating each treatment.

Subjects were fasted (food and beverage) from 10:00 pm the night before their allocated treatment until 4 hr after their medication. Except for 180 ml of water taken with their medication at zero time, no water was permitted for 1 hr before or for 2 hr after dosing; at all other times, it was taken *ad libitum*. Smoking was permitted if it was the usual custom of a subject. Volunteers remained ambulatory during each sampling day, not engaging in strenuous or athletic activities.

Each volunteer received four of the following five 12-mg oral doses of methylprednisolone in crossover fashion, utilizing a double Latin square design (details are summarized in Table I):

Treatment A—Three 4-mg methylprednisolone tablets at zero time, lot 1[1].

Treatment B—Three 4-mg methylprednisolone tablets at zero time, lot 2[2].

Treatment C—Three 4-mg methylprednisolone tablets at zero time, lot 3[3].

Treatment D—Fifteen milliliters of an aqueous suspension of 4 mg of methylprednisolone/5 ml at zero time.

Treatment E—Three 4-mg methylprednisolone tablets at zero time, lot 1[1].

Lots of tablets tested were manufactured from different lots of bulk drug and were of typical production size. Tablets used in the study were randomly selected from one bottle for each lot. Whereas Treatments A–C represented different lots of methylprednisolone tablets, Treatments A and E were the same lot. Table I shows that Treatments A–C were each administered to 20 subjects. Treatment D was given to eight subjects, while Treatment E was given to 12 subjects.

[1] C. T. Medrol, 4 mg, lot 278ER, The Upjohn Co., Kalamazoo, MI 49001.
[2] C. T. Medrol, 4 mg, lot 778ER, The Upjohn Co., Kalamazoo, MI 49001.
[3] C. T. Medrol, 4 mg, lot 423ES, The Upjohn Co., Kalamazoo, MI 49001.

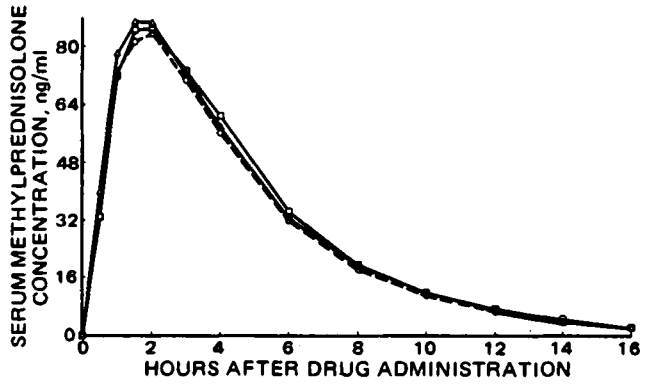

Figure 1—*Average serum methylprednisolone levels after oral administration of a 12-mg dose of three different lots of 4-mg tablets. Key:* □—□, *Treatment A;* O- - -O, *Treatment B; and* △—△, *Treatment C.*

During each phase of the study, all subjects were given two tablets of dexamethasone, 0.75 mg[4]. One tablet was taken 9 hr before and the second tablet was taken 9 hr after methylprednisolone administration. Dexamethasone was given to reduce the level of endogenous cortisol to preclude its interference in the radioimmunoassay for methylprednisolone. Dexamethasone itself does not cross-react with the radioimmunoassay antibody for methylprednisolone (2).

Blood samples were drawn at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 14.0, and 16.0 hr after each methylprednisolone treatment. Serum was harvested from each specimen, frozen, and kept in a frozen state until assayed for methylprednisolone by the radioimmunoassay (2).

RESULTS

Average serum methylprednisolone concentrations and related bioavailability parameters obtained from the 20 subjects, each having received three different lots of methylprednisolone as 12-mg oral doses, are shown in Table II. A summary of the statistical evaluation of the data (see *Appendix*) is given in Table II. No significant differences among treatment average values were found at any sampling time.

Figure 1 clearly shows that the average time courses in serum of methylprednisolone from the three lots tested were superimposable, suggesting identical rates and extents of drug absorption. The correspondence among the averages of individual peak serum concentrations and the times of their occurrence, average areas under the individual serum concentration–time curves through 16 hr and through infinity, and the averages of individual half-lives support this premise. These results indicate lot-to-lot uniformity in methylprednisolone bioavailability. Further evidence for this conclusion comes from Table III, which shows that the ranges and coefficients of variation among the bioavailability parameters listed in Table II were similar.

Methylprednisolone variability can also be evaluated by comparing between-lot variability in bioavailability to the variability within a given lot. This evaluation was accomplished using serum methylprednisolone levels from the 12 subjects who each received Treatments A–C and E and simultaneously verifying the equivalency of variances within lots and the equivalency of covariances between lots. Results of the statistical analysis for the area under the curve, peak maximum, time to peak, and half-life are summarized in Table IV; details are given in the *Appendix*. All of the statistics were not significant at the 5% level. Therefore, within-lot and between-lot uniformities in methylprednisolone bioavailability apparently were similar, suggesting that the manufacturing process had little to do with the observed variability associated with the temporal change of methylprednisolone in serum.

On the assumption that Treatments A and E were identical, a comparison can be made of the inter- *versus* intrasubject variability in methylprednisolone bioavailability. This comparison was accomplished for the parameters peak maximum, time to peak, area under the curve through infinity, and half-life utilizing statistical methodology that permitted an estimate and confidence interval for the ratio of between-subject to within-subject variability. According to the method (see *Appendix*), if the left end-point of the confidence interval exceeds unity, then intersubject variability is greater than intrasubject variability, the

[4] Decadron, Merck Sharp and Dohme, West Point, Pa.

Table II—Average Methylprednisolone Serum Concentrations and Related Bioavailability Parameters for the 20 Subjects Who Each Received Three Lots of Methylprednisolone [a]

Parameter	Treatment A	Treatment B	Treatment C
Average serum levels of methylprednisolone, ng/ml, at:			
0.5 hr	33.9	32.8	40.7
1.0 hr	73.1	72.8	77.8
1.5 hr	85.4	81.4	86.7
2.0 hr	85.2	82.4	86.1
3.0 hr	73.1	69.9	71.9
4.0 hr	60.4	55.4	57.4
6.0 hr	34.1	31.5	32.3
8.0 hr	19.2	17.9	18.7
10.0 hr	11.8	11.0	11.7
12.0 hr	7.13	6.62	6.76
14.0 hr	4.33	3.40	3.57
16.0 hr	2.02	1.81	1.85
Average of individual peak serum concentrations, ng/ml	91.2	91.7	94.2
Average time of individual peak serum concentrations, hr	1.84	1.66	1.54
Average area under individual serum concentration–time curve[b], ng/ml × hr			
Through 16 hr	479	451	473
Through infinity	489	462	480
Half-life, hr[b]	2.63	2.61	2.59

[a] Comparisons were made using an approximation to the *t* test by adjusting for unequal variances between Latin squares (see *Appendix*). The mean differences between Treatments A *versus* B, A *versus* C, and B *versus* C were not significant for any parameter at any time. [b] See *Appendix* for calculation method.

Table III—Ranges and Coefficients of Variation among Selected Bioavailability Parameters

	Range						Percent *SD*		
	Treatment A		Treatment B		Treatment C		Treatment	Treatment	Treatment
Parameter	Low	High	Low	High	Low	High	A	B	C
Peak plasma level, ng/ml	58.5	124	64.1	129	61.9	123	18.9	21.1	20.1
Time to peak, hr	1.0	4.0	1.0	3.0	1.0	3.0	48.1	36.6	32.9
Area under curve[a] through 16 hr, ng/ml × hr	247	643	243	662	271	818	22.1	26.3	28.3
Area under curve[a] through infinity, ng/ml × hr	255	655	251	691	278	854	22.0	26.5	28.7
Half-life, hr[a]	2.16	3.14	2.10	3.28	2.21	3.32	10.7	12.8	11.5

[a] See *Appendix* for calculation method.

magnitude of which is given by the value of the ratio. In contrast, if the left end-point of the constructed interval is less than unity, between- and within-subject variabilities are probably similar because between-subject variability is small.

Table V shows that the left end-point of the confidence interval exceeded unity only for the area under the curve through infinity. Therefore, it can be concluded that with respect to the extent of absorption, intersubject variability was about sixfold greater than intrasubject variability. For the absorption rate (as measured by peak maximum and time to peak) and the half-life (an indicator of disposition), inter- and intrasubject variabilities were similar.

Bioavailability estimates relative to an aqueous methylprednisolone suspension were determined for the eight subjects who received this treatment in addition to the three lots of methylprednisolone. Table VI summarizes the individual bioavailability estimates, which were calculated as ratios of the area under the curve through infinity. The only significant differences were between the means $F^A/F^D = 1.04$ *versus* $F^B/F^D = 0.931$. However, since the three lots were already judged bioequivalent based on data from 20 subjects (Table II and Fig. 1), it was possible to conclude that this observed difference based on eight subjects was biopharmaceutically meaningless. A grand bioavailability ratio (based on 24 observations) was calculated. The overall value of 0.990, which was not significantly different from unity, illustrates that methylprednisolone tablets were fully bioavailable relative to an aqueous suspension given orally.

DISCUSSION

A limited number of studies have been published addressing lot-to-lot uniformity in bioavailability. In 1973, Butler (3) compared two lots of a chemically equivalent oxytetracycline product against a recognized standard and found that one lot was clearly bioinequivalent. Subsequently, DiSanto *et al.* (4) demonstrated a pronounced lot-to-lot variability in nitrofurantoin bioavailability; four lots that were tested against an appropriate reference standard exhibited relative bioavailabilities of 6, 27, 80, and 96%. More recently, Stoll *et al.* (5) compared the within-lot uniformities of two digoxin preparations and found one brand to be significantly more variable than the other. Until now, however, no study has addressed both within-lot and between-lot uniformities in bioavailability.

The selection of a five-treatment study design to document the intra- and interlot uniformity in absorption and the relative bioavailability of methylprednisolone tablets was based on a set of rated objectives and a desire to limit the number of phases to four. Therefore, a five-way Latin square study was precluded. Since the most important objective was the demonstration of between-lot uniformity, a decision was made to administer each of three different lots of drug to all 20 subjects (Treatments A–C). More weight was also placed on the intra- to interlot comparisons than on the bioavailability estimate relative to the aqueous suspension. Accordingly, 12 subjects each received one lot of tablets on a second occasion (Treatment E) while eight subjects each received the aqueous suspension (Treatment D). The overall design was, in essence, a combination of two Latin squares, referred to herein as a double Latin square. As shown in Table I, the eight-subject square was used to estimate bioavailability relative to the aqueous suspension, the 12-subject square was used to estimate intra/inter effects, and the two squares were combined to estimate between-lot uniformity in bioavailability.

The assessment of intrasubject *versus* intersubject variability required the assumption that Treatments A and E were identical. Since they represented tablets from the same lot of drug and since *in vitro* physicochemical tests (such as dissolution rate, disintegration time, and content uniformity) indicated that the lots were homogeneous, these assumptions appeared to be valid. In addition, the statistical methodology requires that period effects normally tested for during individual Latin square analyses of variance be absent or ignored if present. No such effects were observed in the 12-subject A, B, C, E square[5], facilitating the statistical evaluation. A future publication will deal with a design that can accommodate order effects without biasing the statistical conclusions.

In evaluating the statistical results of a comparison of intersubject *versus* intrasubject variability, the magnitude of the variance and resulting coefficient of variation for the tested bioavailability parameter should be considered since they are measures of the sum of the two variations. Where within-subject and between-subject variabilities are

[5] Data available from the authors upon request.

Table IV—Comparison of Between-Lot and Within-Lot Variabilities in the Bioavailability of Methylprednisolone for 12 Subjects Receiving Treatments A–C and E[a]

Parameter	Square Root of Common Variability: Within-Lot Variance	Square Root of Common Variability: Between-Lot Covariance	χ-Square Statistic (8 *df*)
Area under curve[b] through infinity	117	97.5	11.3
Peak maximum	19.4	13.7	3.40
Time to peak	0.75	−0.3[c]	8.76
Half-life[b]	0.26	0.20	9.09

[a] No significant differences were found. [b] See *Appendix* for calculation method. [c] Negative value probably due to sampling variation.

similar and the coefficient of variation is sizable (*e.g.*, ~50%), large intrasubject variations would be anticipated, suggesting that clinical failure may occur for a given segment of the patient population. Both disposition and absorption parameters should be evaluated since the former is drug related while the latter is product related. Accordingly, a less variable drug or less variable product might be dictated.

For the case of a large coefficient of variation where intersubject variability is significantly different than intrasubject variability, an unbiased estimate of the variance ratio of between-subject to within-subject data should be calculated so that the magnitude of the intrasubject contribution can be determined. Its therapeutic effect can, therefore, be evaluated to permit a more rational decision of product substitution. In contrast, when the coefficient of variation is small (25%, as observed here for the area under the curve), loss of therapy or untoward reactions due to the variability of a particular product would not be anticipated regardless of whether intersubject and intrasubject variabilities were significantly different[6]. A future report will address the influence of variability of bioavailability assessment as it relates to product interchangeability.

In summary, the lot-to-lot uniformity and relative bioavailability of methylprednisolone tablets along with an estimate of intrasubject and intersubject variations have been documented in humans. Based on the uniformity between lots and within lots, the small intersubject and intrasubject variations in absorption, and the fact that, on the average, tablets were fully bioavailable relative to an aqueous drug suspension, it can be concluded that the dosage form tested was optimal with respect to bioavailability.

APPENDIX

Comparisons among Treatments A–C—Data from two Latin squares were combined with equal weight to yield comparisons of A *versus* B, B *versus* C, and A *versus* C. Since the residual variations differed, for the most part, between the two squares[5], an approximation to the t statistic was employed:

$$t' = \frac{\bar{d}}{S_{\bar{d}}} \qquad \text{(Eq. A1)}$$

where:

$$\bar{d} = \frac{\bar{d}_1 + \bar{d}_2}{2} \qquad \text{(Eq. A2)}$$

$$\bar{d}_i = \bar{x}_i^A - \bar{x}_i^B; \bar{x}_i^A - \bar{x}_i^C; \bar{x}_i^B - \bar{x}_i^C \quad (i = 1, 2) \qquad \text{(Eq. A3)}$$

and:

$$S_{\bar{d}} = \left(\frac{S_1^2}{2n_1} + \frac{S_2^2}{2n_2}\right)^{1/2} \qquad \text{(Eq. A4)}$$

where $\bar{x}_i$ represents a given average of individual observations from either square 1 ($i = 1$) or square 2 ($i = 2$), with the superscripts referring to a specified treatment within each square; S_i^2 refers to the residual mean square from the analysis of variance; n_i is the number of subjects; and the subscripts refer to either square 1 or square 2.

The value of t_c' such that larger observed values are judged significant is calculated using Eq. A5:

$$t_c' = \frac{w_1 t_1 + w_2 t_2}{w_1 + w_2} \qquad \text{(Eq. A5)}$$

[6] Care should be exercised in interpreting data having a small coefficient of variation resulting from data with a large variance.

Table V—Intersubject *versus* Intrasubject Variability in Methylprednisolone Bioavailability for 12 Subjects Receiving Treatments A–C and E

Parameter	95% Confidence Interval for B/W[a]	B/W[b]
Area under curve[c] through infinity	$1.76 \le B/W \le 20.1$	5.9
Peak maximum	$0.328 \le B/W \le 3.74$	—
Time to peak	$0.033 \le B/W \le 0.379$	—
Half-life[c]	$0.216 \le B/W \le 2.46$	—

[a] Calculated according to Eq. A13. [b] Calculated according to Eq. A12 only where left end-point of 95% confidence interval exceeded unity. [c] See *Appendix* for calculation method.

where t_i is the critical values for the ith square with $(n_i - 2)(k - 1)$ degrees of freedom, $w_i = S_i^2/2n_i$, and k is the number of treatments (6).

For purposes of testing, t_c' need not be calculated if $t' < \min(t_1, t_2)$ or $t' > \max(t_1, t_2)$.

Comparison of Between-Lot and Within-Lot Variabilities—To compare between-lot and within-lot variabilities, the equality of within-lot total variance and between-lot covariance must be simultaneously tested.

The covariance matrix, S, for the 12 subjects who each received Treatments A–C and E is first computed, permitting the overall average variance and the overall average covariance to be estimated. The average covariance matrix, S_0, is then obtained by setting all treatment variances equal to the overall average variance and all treatment covariances equal to the overall average covariance.

Then, according to Box (7):

$$M = -(n - 1) \ln(\det S / \det S_0) \qquad \text{(Eq. A6)}$$

$$C = \frac{k(k+1)^2(2k-3)}{6(n-1)(k-1)(k^2+k-4)} \qquad \text{(Eq. A7)}$$

$$f = \frac{k^2 + k - 4}{2} \qquad \text{(Eq. A8)}$$

where n is the total number of subjects, k is the number of treatments, $\det S$ is the determinant of the matrix S, $\det S_0$ is the determinant of the matrix S_0, and f is the computed degrees of freedom.

Under the null hypothesis of simultaneous equalities of variances and covariances, the following X^2 statistic can be computed whose sampling distribution is approximated by a χ-square with degrees of freedom f:

$$X^2 = (1 - C)M \qquad \text{(Eq. A9)}$$

Comparison of Inter- *versus* Intrasubject Variability—Between-subject variability was estimated under the assumption that Treatments A and E were identical. Period effects were not present.

Table VI—Individual Bioavailability Estimates for Methylprednisolone Tablets Relative to an Aqueous Methylprednisolone Suspension for Eight Subjects Receiving Treatments A–D

Subject	Bioavailability Ratios[a] F^A/F^D	F^B/F^D	F^C/F^D
1	0.775	0.762	0.954
2	0.955	0.849	0.894
4	0.991	0.806	0.844
5	1.11	0.988	0.932
8	1.06	0.966	1.04
17	1.11	0.969	1.16
19	1.28	1.16	1.04
20	1.04	0.946	1.04
Mean	1.04	0.931	1.00
SD, %	14.0	13.4	11.4
Level of significance between pairs[b]			
F^A/F^D *versus* F^B/F^D	$p = 0.001$		
F^A/F^D *versus* F^C/F^D	NS		
F^B/F^D *versus* F^C/F^D	NS		
Grand mean = 0.990[c]			
Grand percent *SD* = 13.3			

[a] Individual ratios of areas under the curve through infinity for tablet lots (Treatments A–C) divided by aqueous suspension (Treatment D). See *Appendix* for calculation method. [b] Comparison was made using a paired t test. [c] Not statistically significantly different from unity using t test.

Then:

$$\text{between-subject sum of squares} = \sum_{i=1}^{12} \frac{S_i^2}{2} - \frac{\left(\sum_{i=1}^{12} S_i\right)^2}{24} \quad \text{(Eq. A10)}$$

with 11 degrees of freedom, $S_i = X_{iA} + X_{iE}$, and:

$$\text{within-subject sum of squares} = \sum_{i=1}^{12} \frac{(X_{iA} - X_{iE})^2}{2} \quad \text{(Eq. A11)}$$

with 12 degrees of freedom.

In Eqs. A10 and A11, X_{iA} and X_{iE} refer to individual subject data following Treatments A and E, respectively.

An estimate of the ratio of between-subject variability to within-subject variability is given by Eq. A12:

$$B/W = \frac{MS_{\text{between}}}{MS_{\text{within}}} \times \frac{1}{k} - \frac{1}{k} \quad \text{(Eq. A12)}$$

when MS_{between} and MS_{within} represent the between-subject mean square and within-subject mean square, respectively, obtained by dividing the appropriate sum of squares by the corresponding number of degrees of freedom; and k is the number of treatments.

The 95% confidence interval for B/W is given by:

$$\text{prob}\left[\frac{MS_{\text{between}}}{kMS_{\text{within}}} \times \frac{1}{F_{0.975}(11,12)} - \frac{1}{k} \le B/W \le \frac{MS_{\text{between}}}{kMS_{\text{within}}} \times \frac{1}{F_{0.025}(11,12)} - \frac{1}{k}\right] = 0.95 \quad \text{(Eq. A13)}$$

If the left end-point of the constructed interval exceeds unity, between-subject variability is considered greater than within-subject variability, the magnitude of which is given by Eq. A12.

Area under Curve and Half-Life Estimation—The area under the curve (AUC) was calculated for each subject following each treatment by the following equation:

$$AUC_\infty = AUC_T + \hat{C}_{T/\beta} \quad \text{(Eq. A14)}$$

where AUC_∞ is the area under the curve through infinity, AUC_T is the area estimated by the trapezoidal rule up to time T, T is the last sampling time (usually 16 hr) when the observed concentration was above the sensitivity limit of the radioimmunoassay, β is the apparent elimination rate constant obtained by the method of least squares in the terminal log-linear phase, and $\hat{C}_T$ is the estimated serum concentration at time T observed by use of the exponential equation that defines the log-linear region.

The half-life ($t_{1/2}$) was estimated by the equation:

$$t_{1/2} = 0.693/\beta \quad \text{(Eq. A15)}$$

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NOTES

Separation of Penicillin G Potassium and Its Degradation Products Using High-Pressure Liquid Chromatography

W. A. VADINO *x, E. T. SUGITA, R. L. SCHNAARE, H. Y. ANDO, and P. J. NIEBERGALL ‡

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*Present address: Pharmaceutical Division, Ciba-Geigy Corp., Summit, NJ 07901. ‡Marion Laboratories, Kansas City, MO 64141.

Abstract □ A high-pressure liquid chromatographic technique was developed for the separation of penicillin G potassium and several of its decomposition products. The method utilized a buffered acetonitrile–phosphate mobile phase on a reversed-phase C_{18} column. Separation of penicillin G potassium and six degradation products was attained within 25 min.

Keyphrases □ Penicillin G potassium—analysis, high-pressure liquid chromatography, separation from degradation products □ High-pressure liquid chromatography—analysis, penicillin G potassium, separation from degradation products □ Antibacterial agents—penicillin G potassium, high-pressure liquid chromatographic analysis, separation from degradation products

The separation of penicillin G from mixtures of penicillin or related decomposition products was reported previously (1–7). Separation methods include TLC (1–3), GLC (4, 5), and high-pressure liquid chromatography (HPLC) (6, 7). Continuing interest in this area is due in part to ongoing efforts to identify the causative agents in penicillin allergy. Most degradation products formed during penicillin G hydrolysis can elicit an allergic response (8).

This report describes an HPLC technique which separates penicillin G potassium and six decomposition products within 25 min.

EXPERIMENTAL

Materials—Penicillin G potassium[1] and D,L-penicillamine[2] were obtained commercially and used as received. Benzylpenicilloic acid, benzylpenillic acid, benzylpenilloic acid, and benzylpenamaldic acid were prepared using standard procedures (9–11). Acetonitrile[3] was spectral

[1] Lot W732511, Wyeth Laboratories, West Chester, Pa.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Matheson, Coleman and Bell, Norwood, Ohio.

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