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Title: THE EFFECT OF FOOD AND TABLET AGE ON THE RELATIVE BIOAVAILABILITY AND PHARMACODYNAMICS OF TWO TOLBUTAMIDE PRODUCTS

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James W. Ayres, Ph.D.

The relative bioavailability and pharmacodynamics of tolbutamide from two different commercially available tablet products has been evaluated in healthy subjects in a single dose cross over study. Products were aged by exposing tablets to 98% relative humidity for 3 days at ambient temperature which was found to differentially affect both rate and extent of absorption for the two products. Differences were reflected by log AUC, peak concentration, mean absorption time, and mean residence

time. The aged inovator's product produced statistically significantly higher serum tolbutamide concentrations for the first 8 hours post dosing and a greater glucose depression than the aged generic product. Administration of unaged tablets with food produced differences in the rate of absorption manifested in time to peak, peak concentration, and mean absorption time which resulted in statistically significantly higher serum tolbutamide concentrations for the first 3 hours post dosing. Administration with food produced a 14% greater reduction in glucose concentration for the inovator's product than for the generic product at 40 minutes post dose. Statistical moment theory, bivariate distribution plots, across study comparisons, non-parametric trend analysis techniques and linear regression were utilized in data analysis. Results of this study demonstrate that the two commercially available products do not respond equally under the conditions investigated.

The Effect of Food and Tablet Age on the Relative Bioavailability and Pharmacodynamics of Two Tolbutamide Products

by

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Typed by Stephen Carl Olson

I dedicate this thesis to my wife, Stacey.
Her love, understanding, encouragement, and
incredible patience may be found on every page.

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THE EFFECT OF FOOD AND TABLET AGE ON THE RELATIVE BIOAVAILABILITY AND PHARMACODYNAMICS OF TWO TOLBUTAMIDE PRODUCTS

INTRODUCTION

Tolbutamide is an orally administered sulfonylurea hypoglycemic agent used in the treatment of maturity onset diabetes mellitus and has been identified as a drug whose clinical efficacy may be compromised by poor bioavailability, particularly in cases of product substitution (1,2,3). The bioavailabilty profiles of certain tolbutamide tablet formulations, however, have been assumed to be equivalent such that these products are considered interchangable (4). Studies which have supported this assumption have involved single dose administration under fasting conditions (5,6). Prior to making the assumption of bioequivalence for this or any other multi-source drug product the behavior of the various formulations should be evaluated under conditions which more accurately reflect those under which the drug is normally administered. Two factors which might affect performance of tolbutamide formulations are food and tablet age.

Recent reviews (7,8) of the effect of food on drug absorption have summarized cases in which drug bioavailability has been enhanced, retarded or delayed, or not affected by concomitant intake of a meal. Previous studies have demonstrated that concurrent injestion of a meal with tolbutamide does not affect

it's subsequent bioavailability in diabetics (9) and in healthy volunteers (10). Only one formulation of tolbutamide was studied in each case, however, and the question of bioequivalence of two or more formulations was left unanswered. Food should continue to be suspected as affecting tolbutamide bioavailability in a formulation specific manner since it has been shown that the sulfonylurea glipizide, with physical chemical characteristics similar to those of tolbutamide, is more rapidly absorbed, and it's blood glucose reducing effect greater if the drug is taken before meals than if it is taken with meals (11,12).

A correlation has been demonstrated between *in vitro* dissolution rate for tolbutamide formulations and serum tolbutamide concentration (bioavailability) and degree of physiologic response (13). Exposure to high degrees of relative humidity has been proposed to "age" tolbutamide tablets and such "aging" has been shown to retard the *in vitro* dissolution of tolbutamide tablets (14). This effect has been shown to be formulation specific with some products being affected to a greater degree and others to a much smaller degree (14,15). Because exposure to humidity enhances differences in *in vitro* dissolution for tolbutamide products and these same differences appear to affect bioavailability of and physiologic response to tolbutamide, it is possible that

humidity aging would enhance product differences in bioavailability to the point of physiological significance. While exposure to humidity has been used to artificially age tablets in the studies cited above as well as in the present one, it is not hard to imagine the conditions as simulating those existing during typical storage in a bathroom or over a kitchen sink.

METHODS

Dissolution - Products were stored in tightly closed, opaque containers in the dark at room temperature and tested using the United States Pharmacopeia (USP) paddle-stirrer method(16). Samples were collected for six tablets of each lot at 10, 20, and 30 minutes for the unaged tablets and for three tablets of each lot at 10, 20, 30, 60, 120, 180 and 240 minutes for the humidity aged tablets. Dissolution samples (5.0 ml) were collected with replacement using temperature equilibrated dissolution liquid. Samples were filtered and diluted prior to assay for tolbutamide with the USP ultraviolet procedure(17) at 226 nm. A seven point standard curve and linear regression analysis were used in the determination of unknowns rather than the USP suggested single point slope method(17).

Humidity Aging - Water vapor pressure in a closed tank was manipulated using saturated salt solutions(18). Tablets aged for use in the present investigation were subjected to 3 days (72 hours) of 98% relative humidity at ambient temperature. Standard all-glass aquariums[1] (50cm long, 26cm wide, 30cm high) with glass covers were used as humidity tanks. A saturated solution of potasium sulfate[2] was prepared in deionized, distilled

water and placed in the bottom of the tank to a depth of 2 to 3 cm (approximately 2.6 liters). A glavanized rack was placed in the tank so as to hold aluminum foil lined Petri dishes 7 cm above the surface of the solution. Air circulation was maintained within the tank by means of a small electrical fan. Humidity within the chamber could be calculated accurately in terms of the specific salt solution used and the temperature maintained(18). It was also monitored using a wet and dry bulb (Mason type) hygrometer[3]. No attempt was made to regulate the temperature within the tank as temperature variability within the laboratory was small and temperature dependence of relative humidity using potasium sulfate solutions is low(18). The tank was made airtight by the use of foam strips impregnated with petrolatum as a seal between the glass cover and the aquarium.

Tablets were subjected to the aging process by placing them in aluminum foil lined Petri dishes without covers, taking care that no tablet touched another. The tank was then sealed shut and not opened until the end of the aging period. Humidity and temperature were monitored daily.

Clinical Design and Procedures - Two commercially available tablet formulations of tolbutamide from two different manufactures were investigated under two different sets of environmental conditions (Table I).

Table I - Design for Administration of Tolbutamide Tablets to Subjects.

Group	Phase (week)			
	I(1)	II(2)	I(3)	II(4)
I	A[a]	B[b]	C[c]	D[d]
II	B	A	D	C

a. Orinase, 500 mg(Upjohn Lot #495EP) administered with a standard meal. b. Tolbutamide, 500 mg(Mylan Lot #595-215) administered with a standard meal. c. Orinase, 500 mg(Upjohn Lot #495EP) which had been exposed to 98% relative humidity for three days and then administered under fasting conditions. d. Tolbutamide, 500 mg(Mylan Lot #595-215) which had been exposed to 98% relative humidity for three days and then administered under fasting conditions.

Each set of treatments was administered as a two by two repeated measures latin square(19) with 8 subjects in each group (Table I). Subject numbers (1 to 16 for the food study and 17 to 32 for the aged tablet study) were randomly assigned to the two groups independently for each treatment set. Subjects were then assigned numbers, 1 to 16 for the food study and 17 to 32 for the aged tablet study, sequentially by the order in which they entered the studies. Of the sixteen subjects that participated for the first two treatments (administration with food), fourteen also participated for the second two treatments (administration following humidity aging).

All 18 subjects underwent comprehensive physical examinations and provided medical histories prior to inclusion in the study. Occasionally, laboratory parameter values for individual subjects would fall outside of normal[4] range. Collection of post-prandial blood samples was invariably the cause of deviation from parmater normal ranges. In these cases the individual was allowed to participate in the study only in the physician performing the physical examination deemed the individual healthy. Criteria collected in each individual's medical history and used for exclusion included the presence of active peptic ulcers, tuberculosis or psychosis, recent myocardial infarction,

therapy with an enzyme inducing agent within 30 days or any other medication within 7 days of study initiation and a history of intolerance to sulfa base drugs. All subjects were non-obese[5] males and ranged in age from 20 to 34 years.

Nine hours prior to dosage for each phase of the study subjects ate a snack consisting of a cupcake[6] and a glass of milk. Following the snack subjects fasted until time of dosing. Each treatment was taken with six ounces of water immediately following collection of a zero-hour blood sample. Following administration of Treatments A and B, subjects were required to ingest a standard breakfast consisting of approximately 800 calories (41% carbohydrate, 18% protein, 41% fat) prepared by a hospital dietician. Following administration of Treatments C and D, subjects continued to fast until 4 hours post dosing. Following the four hour blood sample food and beverages were taken ad lib for all four treatments. No effort was made to limit consumption of foods or beverages (except during the fasting periods) or smoking if it was the usual practice of the subject to do so. Alcoholic beverages were not allowed during the study periods. Blood samples were drawn just prior to dosing and at 20 min, 40 min, 60 min, 90 min, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 8 hr, 12 hr, 16 hr, and 24 hr post dosing. Serum was

harvested as soon as possible after allowing for clot formation, immediately frozen (-60° C), and kept frozen until assayed.

Serum tolbutamide assay - Tolbutamide concentration in serum was determined by a specific high pressure liquid chromatographic (HPLC) assay (20,21). Serum (.5ml) was buffered with a pH 4.4 acetate buffer (2.0 ml) and spiked with propyl paraben[7] (0.5 ml of a 1.0 µg/ml solution) as an internal standard. This aqueous solution was extracted in 50 ml Erlynmeyer flasks with 5% tetrahydrofuran[8] in Hexane[9] (20) (15 ml) for 15 minutes on an Eberbach shaker[10]. Approximately 10 ml of the organic layer was transfered to 15 ml conical test tubes and evaporated to dryness in vacuo at 40° C. The residue was reconstituted with HPLC grade methanol[11] (100 µl) with vortexing. This extract (50 µl) was loaded by automatic injector[12] onto an octadecyl reverse-phase column[13]. The mobile phase (21) 1% Acetic Acid[14] in deionized, distilled water (buffered to pH 5.2 with 2N NaOH) : Acetonitrile[15], (60 : 40) was pumped[16] through the column at 2.0 ml/min and monitored[17] for UV absorbance at 254 nm. Detector output was processed and recorded by a data processor[18] capable of integration. Calibration was achieved by the use of daily standard curves using spiked (USP reference tolbutamide) serum and inverse estimation (22) with the linear regression of response

(tolbutamide area under peak/internal standard area under peak) on tolbutamide concentration (0.0 to 60.0 µg/ml).

All serum samples were assayed for tolbutamide and/or glucose in duplicate. Generally, replication for the tolbutamide assay was performed within a single assay day. Some samples, however, were assayed a third time on a day different from that on which the first two replications were performed. As a result, it was possible to determine the relative magnitudes of inter- and intra-assay day variability. The relative standard deviations for intra- and inter-assay day were 4.9% (n=29) and 5.4% (n=20) respectively. Because the tolbutamide concentrations used for analysis represent the mean of two observations a more appropriate measure of the assay component of the variability associated with the observations would be the relative standard error of the mean which was only 3.5% and 3.8% for intra- and inter-assay day respectively.

Serum glucose assay - Glucose concentration of each serum sample was determined through the use of a commercial, enzymatic, spectrophotometric assay[19]. Kit instructions were followed explicitly except that a seven point standard curve and linear regression analysis were used for calibration and quantification rather than the suggested single point slope method and

each sample was used as its own control. Absorbance was monitored by a flow through cell spectrophotometer[20], and read and recorded by a microprocessor[21].

RESULTS AND DISCUSSION

The mean *in vitro* dissolution profiles for the two formulations under investigation are presented in Figure 1 and Table II. While there are differences in the dissolution rates and in intra-lot dissolution variation (23) for the two products prior to aging, both met compendial standards (Table 2). Following aging with 3 days of 98% relative humidity these formulations are both affected with respect to rate of dissolution. Although the inovator's product demonstrates a depressed dissolution rate following aging it dissolves faster than the unaged generic product. The generic product's slower dissolution rate following aging results in a failure to meet official average minimum tolbutamide dissolution requirements of 70% in 30 minutes for the paddle-stirrer method (16). Tablet to tablet variability in dissolution, expressed as the relative standard deviation (RSD) of the amount dissolved (Table 2), is increased as a result of humidity aging for both products. The variation demonstrated by the generic product following aging is considerably greater than that demonstrated by the inovator's product.

It is assumed that no chemical alteration of tolbutamide takes place during or as a result of the aging process. Hydrolysis of tolbutamide can occur but

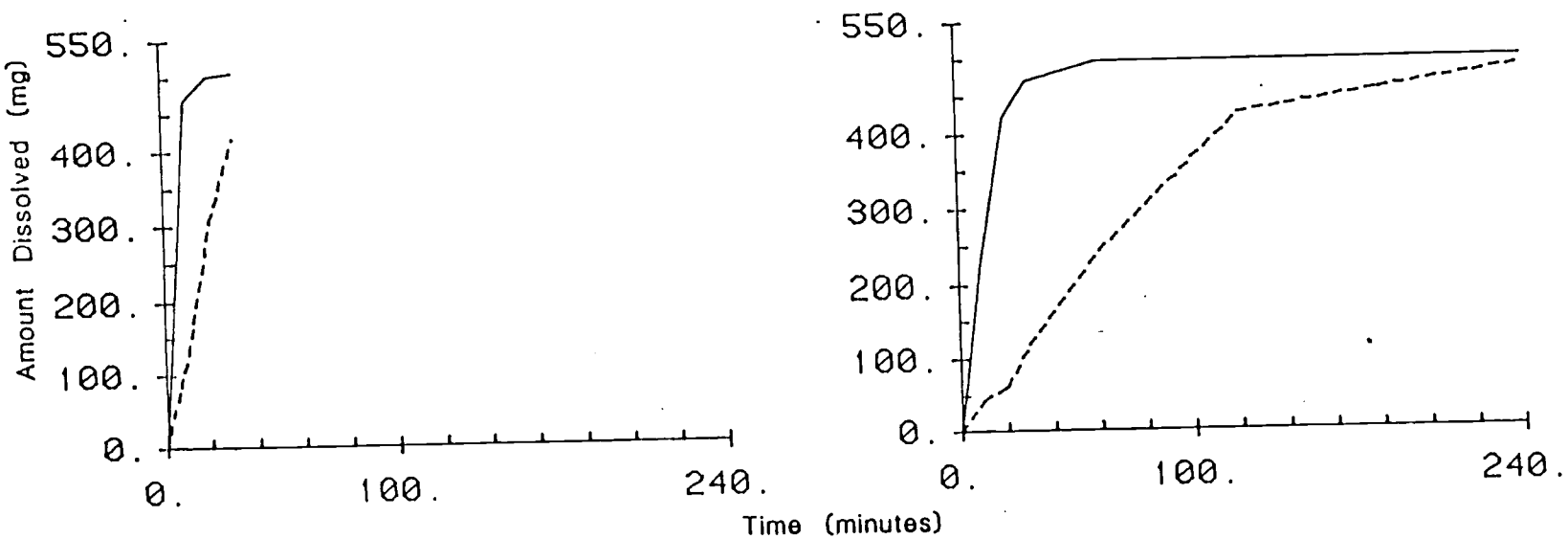

Figure 1 - Mean In Vitro Tolbutamide Dissolution Profiles for Six Tablets of Each Product Prior to Aging (left) and for Three Tablets of Each Product After Aging Through 3 Days Exposure to 98% Relative Humidity (right). Key: Solid line-Orinase, 500 mg(Upjohn Lot #495EP); Dashed line-Tolbutamide, 500 mg(Mylan Lot #595-215).

Table II - *In Vitro* Dissolution of Tolbutamide Before and After Tablet Aging.

Time (minutes)	Mean Amount Dissolved, mg[a]				RSD[b]			
	1[c]	2[d]	3[e]	4[f]	1	2	3	4
10	469.0	130.4	233.4	42.5	4.7	12.3	27.6	63.3
20	503.5	308.1	422.8	63.1	1.6	9.0	6.8	83.4
30	506.1	416.7	472.6	120.2	1.3	3.0	1.3	66.8
60	---	---	498.4	244.4	---	---	0.6	38.1
120	---	---	498.4	426.3	---	---	0.6	8.3
180	---	---	498.4	456.9	---	---	0.6	2.4
240	---	---	498.4	486.4	---	---	0.6	2.1

a. The mean of six tablets for 1 and 2 and of three tablets for 3 and 4. b. Relative Standard Deviation. c. Orinase, 500 mg(Upjohn Lot #495EP). d. Tolbutamide, 500 mg(Mylan Lot #595-215). e. Orinase, 500 mg(Upjohn Lot #495EP) following exposure to 3 days of 98% relative humidity. f. Tolbutamide, 500 mg(Mylan Lot #595-215) following exposure to 3 days of 98% relative humidity.

the extent of degradation is only about 14% when intact tablets are subjected to 90 days of 70% relative humidity at 60° C (24). A similar study (25) in which tablets were powdered prior to aging demonstrated 47% hydrolysis following 60 days of 75% relative humidity at 70° C. Thermal dissociation of tolbutamide has also been demonstrated (26) but the reaction had to be affected at 80° C. in alcoholic solution. The aging conditions for the present study are far less severe and although the USP XIX spectrophotometric technique lacks specificity for intact drug it is highly unlikely that chemical degradation of tolbutamide occured and that the aging phenonenon involved anything but physical degradation of the tablet formulation.

Mean serum tolbutamide concentration *vs.* time curves for sixteen subjects following administration of .5 gram tolbutamide with a meal or after tablet aging are presented in Figure 2 and Table III. The mean serum tolbutamide time courses presented in this figure are deceiving if viewed as "typical" curves for an individual. Figure 3 demonstrates the large amount of variability among subjects in serum tolbutamide concentrations and the prolonged absorption patterns that were frequently present following administration of humidity aged tablets. A large degree of inter-subject variability in tolbutamide clearance was present with

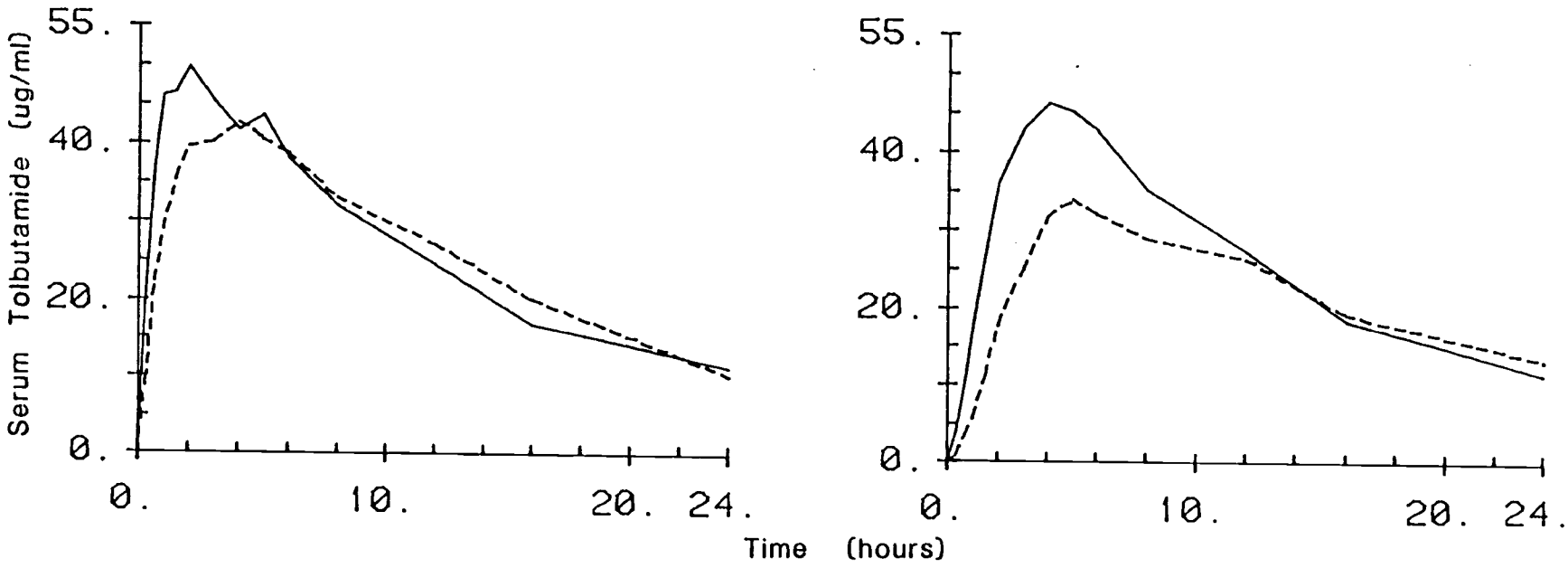

Figure 2 - Mean Serum Tolbutamide Concentration vs. Time Profiles for Sixteen Subjects Following Administration With Food (left) and After Tablet Aging (right). Key: Solid line, Treatments A and C; Dashed line, Treatments B and D. See Table I for Treatment Assignments.

Table III - Summary of In Vivo Results for Two Tolbutamide Products Administered Under Two Sets of Environmental Conditions.

Parameter	Administration with Food			Administration with Aging		
	Response[a]		Sig[b]	Response[a]		Sig[b]
	A	B	p	C	D	p
Serum Tolbutamide Concentration(µg/ml) at:						
0.3 hr	20.6	9.4	.014	3.8	1.0	.024
0.6 hr	39.0	22.4	.005	10.2	3.5	.027
1.0 hr	48.9	29.0	.0006	18.0	5.9	.009
1.5 hr	49.2	35.5	.005	27.3	11.4	.005
2.0 hr	52.9	39.5	.005	36.0	18.0	.002
3.0 hr	48.2	40.2	.023	43.1	25.1	.0001
4.0 hr	44.1	42.7	NS	46.3	31.8	.0001
5.0 hr	46.1	40.4	.023	45.2	34.0	.0004
6.0 hr	39.9	38.6	NS	42.9	32.1	.001
8.0 hr	39.5	33.1	NS	35.4	28.8	.023
12.0 hr	25.4	27.1	NS	27.4	26.3	NS
16.0 hr	16.8	20.1	.005	18.4	19.2	NS
24.0 hr	10.6	10.3	NS	11.5	13.3	NS
Peak Concentration (µg/ml)	60.0	49.7	.001	52.5	38.4	.0001
Peak Time (hr)	2.4	4.1	.005	4.5	5.8	NS
AUC (µg/ml hr)[c]	779.	761.	NS	764.	687.	NS
ln(AUC)	6.56	6.52	NS	6.57	6.45	.047
MRT (hr)[d]	12.1	13.1	NS	13.9	16.4	.011
MAT (hr)[e]	.9	2.0	.007	2.2	4.8	.0008

a. Mean response for sixteen subjects. See Table I for specific study conditions associated with each Treatment.
b. Probability of equivalence as determined by Latin Square ANOVA. NS=Not Significant. c. Calculated by the linear trapezoidal method (see text). d. Calculated as the ratio of area under the first moment curve to AUC.
e. Calculated as MRT minus the reciprocal of the terminal phase decay constant.

Figure 3 - Individual Serum Tolbutamide Concentration vs. Time Profiles for Three Subjects (top, center, bottom) Following Administration of Tablets with Food (left) and After Tablet Aging (right). Key: Solid line, Treatments A and C; Dashed line, Treatments B and D. See Table I for Treatment Assignments.

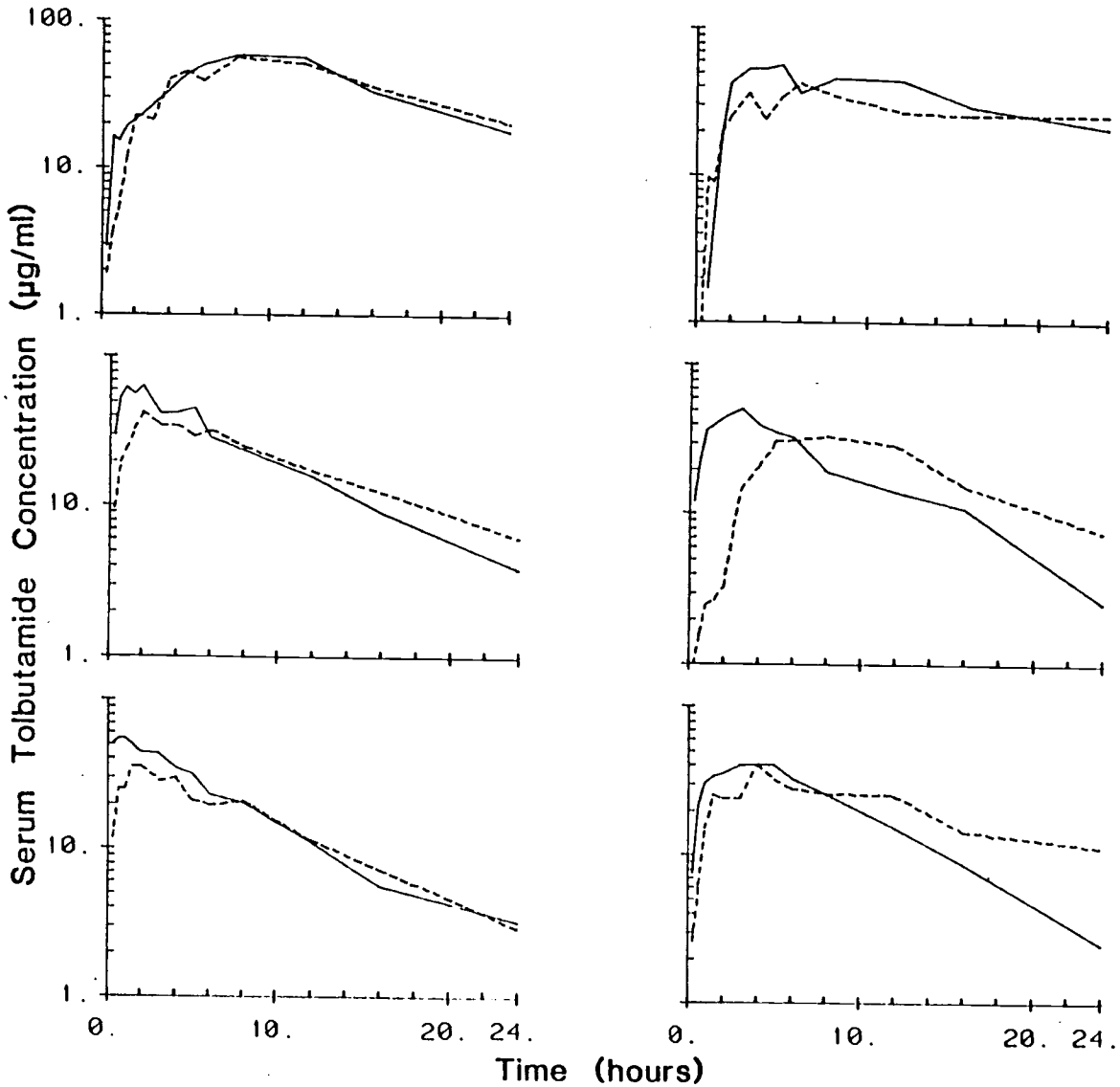
100.
10.
1.
Serum Tolbutamide Concentration (µg/ml)
0.
10.
20.
24.
Time (hours)

Figure 3

eliminatation half-lives ranging from 3.8 to 15.1 hours. Similar variablility in tolbutamide pharmacogenetics has been reported in previous studies(6,27,29).

Bioavailability, defined (29) as the rate and extent to which the drug ingredient is absorbed from the drug product, was determined through the use of six model independent parameters. For each individual's serum tolbutamide curve time to peak, peak concentration, mean residence time (MRT), mean absorption time (MAT) and area under the curve extrapolated to infinity (AUC) were determined. The sixth parameter was the serum glucose time course which will be presented and discussed last. Peak tolbutamide concentrations and times to peak were determined as actual values observed with no attempt to interpolate.

Mean residence time is calculated as the ratio of the zeroth and first moments of the tolbutamide concentration *vs.* time curve and approximates (30,31) the sum of the reciprocals of the absorption and elimination processes:

$$MRT = 1/k_a + 1/\beta . \qquad (1)$$

where k_a represents a quantification (not necessarily first order) of absorption and β is the terminal phase decay constant. Because the rate of elimination is usually much slower than the rate of absorption the major contribution to MRT comes from the elimination

process. As a result true differences in the absorption rate may be masked by the influence of β and it's fluctuations. The statistical power of a test examining differences in the absorption process for two formulations of a drug may be increased over that available when using MRT by utilizing MAT which is defined (30,32) as:

$$MAT = MRT - 1/\beta . \qquad (2)$$

This new parameter, MAT, better reflects the absorption process as the effect of the elimination phase on perception of absorption has been removed. MAT values in Table 3 show differences exist in rate of absorption between the products tested.

Area under the curve was determined as the sum of two segments. The linear trapezoidal equation (33) was used for the entire period of sampling to calculate the first segment. While the log-linear method is more accurate during the terminal phase (33) the method was not employed. In light of the erratic and prolonged absorption (see Figure 3, center right, Treatment D) demonstrated in many individuals the log-linear trapezoidal method would have produced no greater accuracy than the linear method. In addition, it has been shown (33) that when the relative magnitudes of terminal sampling interval and drug half-life are as they are for the present study, the degree of

overestimation introduced through the use of a linear trapezoidal method is small even with well defined terminal phases. The second segment of AUC was that determined by extrapolation of the area from time of last sample to infinity by the use of Equation 3.

$$AUC\Big|_{24}^{\infty} = \hat{C}_{24}/\beta \qquad (3)$$

For those curves that demonstrated poorly defined or non-existant log-linear terminal phases following administration of aged tablets (Treatments C and D) it was necessary to use the average of the β values obtained in the subject following administration of tablets with a meal (Treatments A and B). This β was used along with the actual serum concentration at 24 hours rather than an estimated concentration in calculating extrapolated AUC for prolonged absorption curves (34).

Further evidence of the adequacy of the methods used to approximate AUC for individuals with prolonged absorption following administration of Treatments C and D is demonstrated in Figure 4. In this figure the natural logarithms of AUC produced by the generic tablets (M) are plotted against the natural logarithms of AUC produced by tablets manufactured by the inovator (U). The distribution of AUC departs significantly from normal as evidenced by the Wilk-Shapiro test ($p<.01$) and a test of skewness ($p<.05$). The log transformation

Figure 4 - Bivariate Distribution of log AUC Obtained Following Administration of Tablets With Food (circles) and After Tablet Aging (squares). Numbers within symbols assign response to subject for those subjects that recieved all four Treatments.

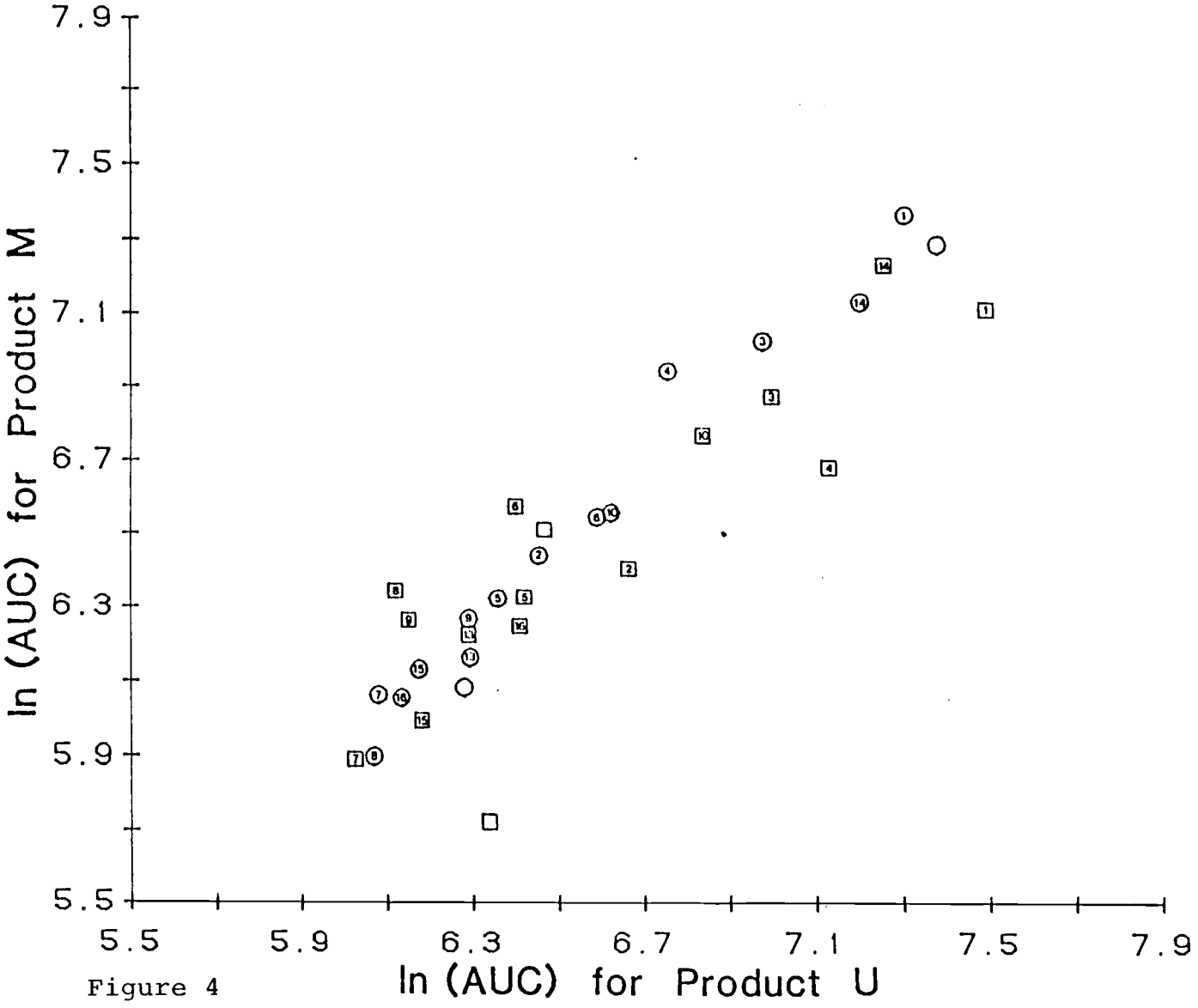
7.9
7.5
7.1
6.7
6.3
5.9
5.5
ln (AUC) for Product M
5.5
5.9
6.3
6.7
7.1
7.5
7.9
ln (AUC) for Product U

Figure 4

renders a distribution for which the hypothesis of normality cannot be rejected by the criteria of skewness or kurtosis or the Wilk-Shapiro test (α=.05). The circles and squares identify the coordinates of pairs of AUCs obtained in individuals following administration of the tablets with food and after aging, respectively. The numbers within the symbols associate a given subject with a given pair of observations. Such a scatter of points represents a bivariate distribution in which the major axis (along the length of the scatter) gives a measure of inter-subject variability in log AUC and the minor axis (through the width of the scatter and perpendicular to the major axis) gives a measure of variablity in response within single subjects. This variation, sometimes called intra-subject variation, arises from many factors including product variability in availability, short term physiologic variation in drug elimination, and assay errors. It is that variation which would be observed if the same formulation were to be given to the same individual many times. Obviously, a time frame must be imposed for this definition to hold as advancing age and/or declining health can alter renal and metabolic clearance and thus bring about a non-random shift in AUC. The time frame to be considered when applying this descriptive definition of intra-subject variance is that of the

study itself. The literature clearly demonstrate that intra-individual tolbutamide clearance is not subject to weekly fluctuations of a magnitude that would invalidate conclusions based on the approximations made in this study (5,6,9,10,27). Note that differences in the extent of absorption for two treatments poses no problem for this definition or for estimation of intra-subject variability from bivariate plots such as that in Figure 4 as these differences would only shift the distribution vertically down or horizontally left (depending on which treatment exhibited a depressed extent of absorption) without effecting the internal integrity of the distribution. The only assumption that must be made is that extent of absorption for any treatment or formulation is not correlated with clearance for the panel of test subjects. This assumption seems most tenable. Evidence of the adequacy of approximating AUC with previously determined first order decay constants lies in the structure of this bivariate distribution of log AUC. The square symbols numbered 6 and 8 indicate cooridinates for pairs of AUCs obtained in two subjects for which AUC for Treatment D was approximated using the β obtained following administration of Treatment C. Squares numbered 1, 3, 14 and 15 indicate similar cooridinates for which both Treatment C and D AUCs were approximated using the average β obtained following

administration of Treatments A and B (circles with the same respective numbers). Note that on the average intra-subject variation, as measured by the distance separating circles and squares with the same subject number, is no greater or smaller for these subjects in which an approximation was required than that for subjects in which no approximation was needed. Quantitatively, the mean separation for subjects not requiring an AUC approximation is .217±.137 SD (n=8) and that for those subjects for which an approximation was needed is .226 ± .130 SD (n=6). Since intra-subject variability includes a component due to computational errors and approximations one would conclude that the similarity in variability for those subjects which required an approximation of AUC and those that did not would indicate no serious amount of error has been injected into AUC by approximation of β.

A summary of serum tolbutamide concentrations and the bioavailability parameters is presented in Table III. All parameters were subjected to a repeated measures Latin Square Analysis of Variance (37) in which variability was partitioned to allow determination of significance of period as well as treatment effects. The period effect was invariably not significant (α =.05). The probabilities associated with treatment effects are those in Table III. Statistical analysis of

individual parameters reveals significant differences in time to peak (B 69% later than A, p=.005), peak concentration (B 18% lower than A, p=.001) and mean absorption time (B 104% greater than A, p=.007) when the products are administered with food. Significant differences in all three of these parameters indicates an inequivalence in the two products with respect to the rate of *in vivo* dissolution and/or absorption. This inequivalence results in differences in mean serum concentrations of tolbutamide for the two products during the absorption phase with Treatment A yielding significantly higher blood concentrations than Treatment B at each sampling time for the first 3 hours post dosing.

Extent of absorption as measured by AUC appears to be the same for the two products when administered with food. Figure 5 displays a bivariate distribution of log AUC for 16 subjects following administration of the two products with a meal in the present study and for 14 subjects following administration of two different lots of the same products under fasting conditions in a previous study (6). Visual examination of the superimposability of the two sets of observations reveals that not only is extent of absorption similar for these two products whether administered with or without a meal but that intra-subject variability in

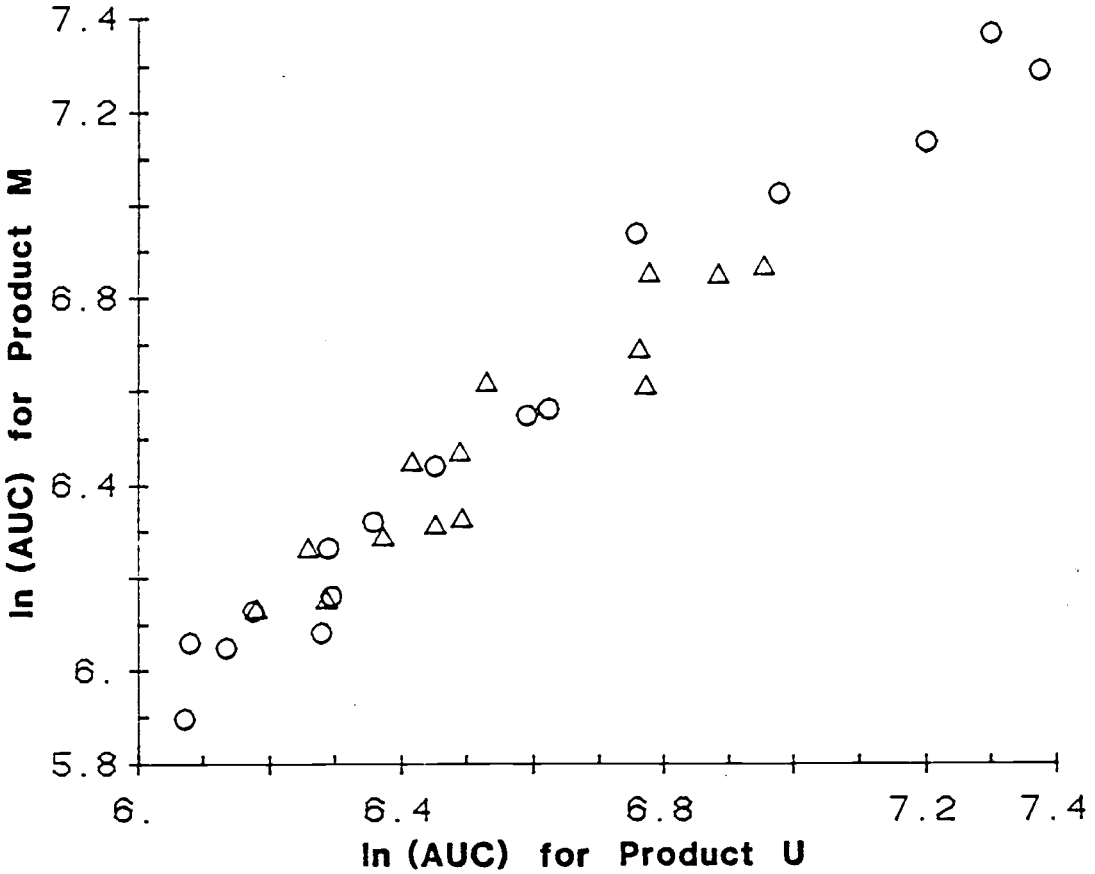

Figure 5 - Bivariate Distribution of ln(AUC) Obtained Following Administration of Tablets with Food (circles) in Present Study and Under Fasting Conditions (triangles) in a Previous Study.

extent of absorption is also the same. Thus, it would appear that food has no differential effect on the extent or intra-subject variability in extent of absorption for these two products.

Examination of parameter differences in Table III obtained following administration of humidity aged tablets reveals significant differences in log AUC (D 10% less than C, p=.047), peak concentration (D 27% lower than C, p=.0001), mean residence time (D 17% longer than C, p=.011) and mean absorption time (D 119% longer than C, p=.0008). These results suggest differences in both rate and extent of absorption for these two products when administered after aging. To determine the effect of approximating AUC on conclusions related to extent of absorption analysis of variance in AUC was carried out using only those 8 subjects for which no approximation of AUC was required. A mean difference in AUC of 15% (D<C) was found to be significant (p=.045). This difference and the level of significance are in good agreement with those obtained above using the full panel of subjects. The effect of these parameter differences on the serum tolbutamide time course is such that Treatment C yields significantly higher serum concentrations than Treatment D throughout the first 8 hours post dosing. Although there is a marginal suggestion that there exists a

difference in extent of absorption for these products after aging, 81% (13 out of 16) of the subjects demonstrated a relative extent of absorption (AUC D/AUC C) of .75 or greater. This result is not the same as that obtained by strict application of the 75/75 rule (35) and should not be interpreted as such since the products are being compared to one another and not to a reference solution or standard. A measure of product variability in extent of absorption may be obtained by other methods, however, which are discussed below.

Figure 6 displays bivariate distributions of log AUC obtained following administration of Treatments A and B (left) and Treatments C and D (right). While inter-subject variability (along the major axis) for the products remains the same whether they are administered with food or after aging, the figure clearly demonstrates a difference in intra-subject variability (through the minor axis) for the two sets of conditions. It is highly likely that this difference is a function of tablet aging rather than food for as pointed out above intra-subject variability in extent of absorption is the same whether these two products are administered with or without food. It is impossible, however, to determine from Figure 6 alone whether increased variability is associated with both products to the same extent, indicating a non-specific aging effect, or with

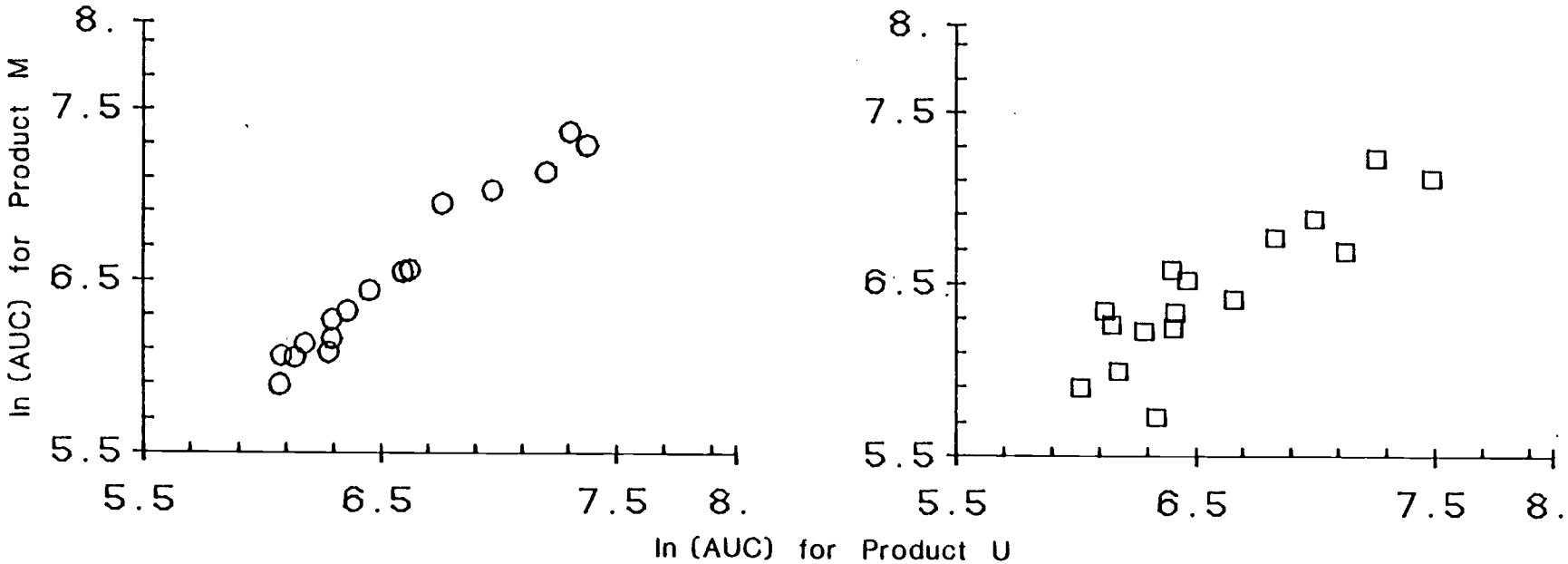

Figure 6 - Bivariate Distributions of ln(AUC) Obtained Following Administration of Tablets With Food (left) and After Tablet Aging (right).

one of the products more than the other, indicating a formulation specific effect on variability in extent of absorption with respect to these two products. Some insight in regards to this question may be obtained through the use of regression analysis.

Normally, linear regression involves an independent variable, plotted on the abscissa, for which values are known without error, and a dependent variable, plotted on the ordinate, for which values are realizations of a random variable with mean linearly related to the independent variable and constant variance estimated by the mean square error (MSE) of the regression for any given value of the independent variable (37). No serious problems are introduced if the independent variable observations are realizations of a random variable as well, as in the present situation of a bivariate distribution of log AUC. All regression coefficients may still yield unbiased estimators of the true population parameters, the only difference being that accuracy of estimation is decreased and MSE is increased (36). The degree of uncertainty and variability associated with a regression in which the independent variable is not known precisly is directly proportional to the degree of uncertainty and variablity associated with the measurement of that independent variable. Thus, the most accurate estimate

of intra-subject variance in extent of absorption for a product will be a MSE obtained from a regression in which the independent variable is known with the greatest accuracy. The logical choice for an independent variable when evaluating intra-subject variation in extent of absorption for aged tablets then is log AUC obtained from the same individual following administration of the tablets with food. This is because unexplained variance (that not attributed to inter-subject differences) when log AUC for one product administered with food is regressed on log AUC for the other administered with food is only 3% of the total variance seen in log AUC (r^2=.969). This 3% represents variability in log AUC that may be attributed to environmental and intra-individual physiologic perturbations and measurement errors. Residual variance following a similar regression using aged tablet bioavailabilities involves 25% of the total variance (r^2=.752). The additional 22% is a result of increased variablility in extent of absorption which may arise in one or the other or both products. Regression of log AUC for aged tablets on log AUC for unaged tablets should reveal, through MSE and r^2, the source of the extra variability. Table IV is a summary of relavent regression parameters. Note that the mean square error obtained following administration of tablets with food

Table IV - Summary of Relevent Estimators Obtained From Various Regressions on Bivariate Distributions of ln(AUC)

Regression[a]	n[b]	MSE[c]	R^2[d]
A on B	16	.006366	.969
B on A	16	.007717	.969
C on D	16	.051783	.752
D on C	16	.046582	.752
C on A	14	.025649	.892
D on B	14	.030859	.827
C on A	8	.022843	.854
D on B	8	.025553	.717

a. Dependent variable on independent variable. b. Number of points (subjects) used in regression. c. Mean Square Error or intra-subject variance (subject to inflation due to imprecision of measure of independent variable). d. Fraction of total variability in dependent variable 'explained' by independent variable.

(A on B and B on A) is significantly smaller than that obtained following administration of aged tablets (C on D and D on C). This is visually apparent in Figure 6. Note also that the two more accurate estimates of MSE for Treatments C and D (those obtained from regressions of C on A and D on B) are smaller than those obtained from regressions involving aged tablet results only and yet are still significantly greater ($F=4.03$ and 4.00 respectively, $p<.001$) than MSE for the products when administered with food. Although the variance for Treatment D is greater than for Treatment C this difference is not significant. These results probably reflect an effect of the aging process on variability in extent of absorption which is not differentially formulation specific relative to these two products. The possibility that increased intra-subject variability was due to the approximation used in estimating AUC for some curves was tested by regressing C on A and D on B using only those 8 subjects which required no approximation. These regressions are the last two of Table IV. The similarity of MSE for these two regressions to the MSE obtained from regressions of C on A and D on B using the full set of 14 subjects indicates that the β approximation was not responsible for the major portion of the increase in intra-subject variability.

A greater degree of caution than usual must be maintained when hypothesizing causal relationships that involve both the food and aging information in this study as such interpretations lack statistical support. One can never validate whether a phenomenon observed following administration of the aged tablets that was not observed when the tablets were administered with food was a result of the aging process or lack of concomitant intake of food. By the same token observations unique to results obtained following administration with food may not, with sound statistical backing, imply food rather than lack of aging as the cause. The degree of caution maintained need not be too great when considering the causal relationship outlined above, however, because strong evidence has been given which does not implicate concomitant food intake as decreasing variability in extent of absorption of tolbutamide.

Figure 7 and Table V present serum glucose data obtained during the first 4 hours post dosing. The only point at which treatment effects were significant at a level of $\alpha = .05$ was at 40 minutes after administration of the tablets with food. At this point Treatment A produced significantly lower serum glucose levels than Treatment B by an average of 14%. This physiologic outcome is in accordance with more rapid absorption and

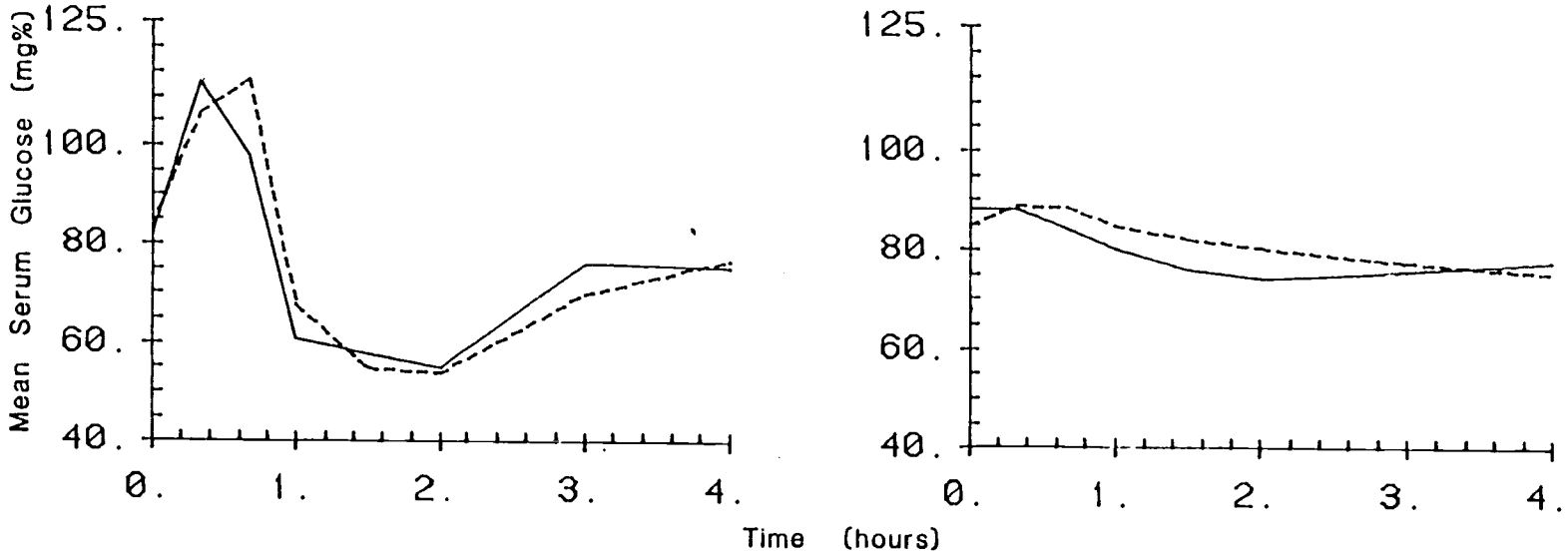

Figure 7 - Mean Serum Glucose Concentration vs. Time Profiles for Sixteen Subjects Following Administration With Food (left) and After Tablet Aging (right). Key: Solid line, Treatments A and C; Dashed line, Treatments B and D. See Table I for Treatment Assignments.

Table V - Summary of Glucose Concentration Response to Two Tolbutamide Products Under Two Sets of Environmental Conditions.

Serum Glucose Level at:	Administration with Food			Administration with Aging		
	Response[a]		Sig[b]	Response[a]		Sig[b]
	A	B	p	C	D	p
0.0 hr	81.8	83.2	NS[c]	86.8	83.5	NS
0.3 hr	112.5	106.0	NS	86.8	87.6	NS
0.6 hr	96.9	113.3	.01	83.3	86.7	NS
1.0 hr	59.8	67.5	NS	78.5	83.3	NS
1.5 hr	56.6	53.7	NS	74.3	80.3	NS
2.0 hr	53.8	53.6	NS	72.6	78.5	NS
3.0 hr	75.4	68.9	.07	73.8	75.6	NS
4.0 hr	73.9	76.0	NS	75.9	73.5	NS

a. Mean response for sixteen subjects in mg%. See Table I for specific study conditions associated with each Treatment. b. Probability of equivalence as determined by Latin Square ANOVA. c. NS=Not Significant.

significantly higher concentrations of drug through the absorption phase following administration of Treatment A. Individual treatment differences in serum glucose at 40 minutes are directly correlated with individual treatment differences in MAT ($p=.0153$) and inversely correlated with individual treatment differences in serum tolbutamide concentration at 40 minutes ($p=.0089$) for these two products when administered with food. While correlation is not synonymous with causality it is strongly supportive, in this case, of a treatment effect and thus a difference in physiologic response to the formulation of these two products when administered with food.

At no point in time do the differences in serum glucose concentration observed following administration of the aged tablets reach statistical significance as determined by parametric methods. Referring the results to the non-parametric runs test (38), however, verifies the visual interpretation of a treatment effect on overall serum glucose profiles ($p < .05$). The reasoning behind this test is that if the two treatments produce equivalent serum glucose profiles then the two curves should cross one another more often in a random fashion. The fact that serum glucose treatment differences are small and do not at any individual sampling time test statistically significantly different following

administration of the aged tablets, even though the treatment differences in serum tolbutamide are greater than those obtained when the products were administered with food, lends support to the hypothesis that the insulin releasing effect of sulfonlyureas is dependent upon blood glucose concentration (39,40). If such a relationship exists, with a given sulfonylurea concentration inducing a greater glucose depression at higher initial glucose levels, then it is quite conceivable that differences observed following administration of tablets with food would be magnified if humidity aged tablets were administered with food. It is also conceivable that the slower absorption rate observed for the humidity aged tablets would require earlier administration of tolbutamide with respect to consumption of the meal in order for therapeutic levels of drug to be present during the glucose peak.

Support for the validity of the approximations made in extrapolating AUC for those subjects in which absorption was prolonged has been given throughout this discussion. The major points that have been made are as follows. Using just those subjects for which no approximation of AUC was required a mean difference in AUC of 15% (C>D) is significant. This difference and significance level are in excellent agreement with those obtained using the full panel of subjects. MAT, which

relies on accurate estimation of AUC, AUMC, and β for a biopharmaceutically sound value shows significant correlation with physiologic response to tolbutamide in a manner and direction consistent with current pharmacologic theory. This correlation is not significantly altered or improved by dropping the data obtained from individuals in which AUC was approximated. The literature clearly demonstrate that intra-individual tolbutamide clearance is not subject to weekly fluctuations of a magnitude that would invalidate conclusions based on the approximations made in this study (5,6,9,10,27). Lastly, intra-subject variability in extent of absorption, as determined by spatial separation in bivariate distributions of log AUC, is the same whether data obtained from subjects in which AUC was approximated are dropped or not.

SUMMARY AND CONCLUSIONS

The effect of concomitant administration of food or tablet aging on the relative bioavailability and relative pharmacodynamics of tolbutamide from two different commercially available tablet products has been evaluated in healthy subjects in a single dose study. Tablets were administered with a standard meal or after tablet aging under fasting conditions in two independent cross-over study designs. Aging was accomplished by exposing tablets to 98% relative humidity for 3 days at ambient temperature. *In vitro* dissolution profiles prior to aging demonstrated rapid dissolution for both products but dissolution profiles obtained after tablet aging exhibited slight retardation of dissolution rate for the inovator's product and a dramatic retardation of dissolution rate for the generic product, with the latter failing to meet official *in vitro* dissolution requirements for tolbutamide.

Tablet aging was found to differentially effect both rate and extent of absorption for the two products investigated with differences being reflected by log AUC (generic 10% smaller than inovator, $p=.047$), peak concentration (generic 27% lower than inovator, $p=.0001$), mean residence time (generic 17% longer than inovator, $p=.011$), and mean absorption time (generic

119% longer than inovator, p=.0008). Effect of these differences on the serum tolbutamide time course was such that the inovator's product produced statistically significant higher serum concentrations with respect to the generic product for the first 8 hours post dosing. The inovator's product produced a statistically significant greater depression in serum glucose concentration than the generic product when the data were referred to nonparametric techniques. It is conceivable that this difference in physiological effect would be magnified during glucose peak concentrations which occur after intake of food since it has been suggested that the effect of sulfonylureas on blood glucose is proportional to glucose concentration.

Food was not found to differentially effect the extent of absorption for these two products but did produce differences in rate of absorption which were manefested in time to peak (generic 69% later than inovator, p=.006), peak concentration (generic 18% lower than inovator, p=.001) and mean absorption time (generic 104% greater than inovator, p=.007). This inequivalence resulted in differences in mean serum concentration of tolbutamide for the two products during absorption with the inovator's product yielding statistically significant higher concentrations than the generic product at all six sampling times occuring during the

first 3 hours after administration. Intra-subject variability in extent of absorption for these two products when administered with food was examined through residual analysis following linear regression of log AUC for one product on log AUC for the other and was found to account for only 3% of the total variability seen in log AUC. This would indicate very uniform behavior by these two products with respect to extent of absorption when administered with food. A differential treatment effect was observed on the serum glucose profiles obtained following administration of the products with food with the inovator's causing a 14% greater reduction in glucose concentration than the generic product at 40 minutes post dose ($p=.01$). Individual treatment differences in serum glucose concentration at this sampling time were positively correlated with individual treatment differences in mean absorption time ($p=.02$) and inversly correlated with individual treatment differences in serum tolbutamide concentration at 40 minutes post dose ($p=.009$).

Results of this study demonstrate that the two commercially available products evaluated do not respond equally under the conditions investigated.

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