teration in the pharmacokinetics of I through a combination of effects including decreased microsomal metabolism and decreased volume of distribution. Furthermore, this study shows that nonmicrosomal hepatic metabolism, such as acetylation, is relatively resistant to pathological insults such as bile duct ligation.

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Bioavailability of Tolazamide from Tablets: Comparison of *In Vitro* and *In Vivo* Results

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Abstract □ The relative bioavailability of tolazamide was determined, in healthy male volunteers, from four different tablet formulations manufactured by direct compaction or granulation processes and the results were compared with *in vitro* disintegration and dissolution values. Serum tolazamide levels were determined by a high-pressure liquid chromatographic method developed in this laboratory. Serum tolazamide levels from the formulation that gave rise to rapid absorption were described by one-compartment model kinetics with a mean absorption half-time of 1.0 hr and an elimination half-life of 4.6 hr. Peak serum levels occurred at 3.3 hr after drug administration. Marked differences were observed in drug bioavailability from the four tablets, and the mean cumulative relative fraction of dose absorbed was 1.0, 0.42, 0.75, and 0.91 from Formulations A, B, C, and D, respectively. The hypoglycemic effect was closely related to serum tolazamide levels. Disintegration times did not predict *in vivo* tolazamide bioavailability. Dissolution rates provided an approximate rank order correlation with *in vivo* absorption but failed to be predictive among formulations. Currently available *in vitro* tests do not accurately predict tolazamide *in vivo* bioavailability characteristics among different formulations and manufacturing processes but may be useful to ensure lot-to-lot uniformity in bioavailability for a given formulation and specific method of manufacture.

Keyphrases □ Bioavailability—tolazamide from tablets, comparison of *in vitro* and *in vivo* results, humans □ Tolazamide—bioavailability from tablets, comparison of *in vitro* and *in vivo* results, humans □ High-pressure liquid chromatography—determination of tolazamide bioavailability from tablets, comparison of *in vitro* and *in vivo* results, humans

The hypoglycemic effect of oral sulfonylureas is related to the size of the dose and to the bioavailability of administered drug to the systemic circulation (1, 2). Large differences in circulating levels of chlorpropamide (3, 4) and tolbutamide (5) have been reported from different generic brands, and the *in vivo* bioavailability of tolbutamide did not correlate well with *in vitro* dissolution rates (6).

Although the sulfonylurea tolazamide has been used clinically since 1965, there appears to be little or no information on its pharmacokinetic or bioavailability characteristics. Therefore, this study was designed to investigate the pharmacokinetics of tolazamide after administration of oral tablets to healthy volunteers and to compare relative *in vivo* bioavailability of different tablets with their *in vitro* disintegration and dissolution rates.

EXPERIMENTAL

Tolazamide Formulations—The four tolazamide tablet formulations selected for study were:

A: Tolazamide 250-mg tablets, marketed Formulation[1]
B: Tolazamide 250-mg tablets, experimental Formulation 1[2]
C: Tolazamide 250-mg tablets, experimental Formulation 2[3]
D: Tolazamide 250-mg tablets, experimental Formulation 3[4]

Formulation A was the commercial brand of tolazamide. Formulations B, C, and D were experimental tablets containing similar excipients as Formulation A but in different quantities to produce different *in vitro* dissolution characteristics. Tablets A and D were wet granulation formulations; B and C were direct compression formulations.

***In Vitro* Studies**—Disintegration times were determined by the official USP XX method for uncoated tablets (7).

Tablet dissolution rates were determined by a rotating paddle procedure. The apparatus[5] consisted of a 1000-ml flask containing 900 ml of 0.05 *M tris*(hydroxymethyl)aminomethane aqueous buffer (pH 7.6) and a paddle stirring rate of 75 rpm. Drug dissolution was monitored by continuously pumping the dissolution medium through a 0.5-mm pathlength flow cell[6] and measuring UV absorbance at 224 nm.

***In Vivo* Studies**—*Subjects*—Subjects[7] were 20 healthy male volun-

[1] Tolinase tablets, Lot No. 901HK, The Upjohn Co., Kalamazoo, Mich.
[2] No. 19356, The Upjohn Co., Kalamazoo, Mich.
[3] No. 19357, The Upjohn Co., Kalamazoo, Mich.
[4] No. 19358, The Upjohn Co., Kalamazoo, Mich.
[5] SPADRA, The Upjohn Co., Kalamazoo, Mich.
[6] Kintrac VII, Beckman Instruments, Fullerton, Calif.
[7] Technical and administrative staff and graduate students.

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Table I—Disintegration Times and Dissolution Rates of Tolazamide Tablets[a]

Tablet	Mean Disintegration Time[b], min (range)	Percent Dissolved in 30 min[c] (range)
A	3.8 (3.0–4.0)	103.9 (100.5–106.3)
B	2.2 (1.8–2.5)	10.9 (9.3–13.5)
C	2.3 (2.0–2.5)	31.6 (26.4–37.2)
D	26.5 (22.5–30.5)	29.7 (20.8–38.4)

[a] $n = 6$. [b] By the method of USP XX (Ref. 7). [c] Dissolution rates in pH 7.6 buffer.

teers between 18 and 38 years of age (mean 26) and weighing between 61.4 and 95.5 kg (mean 74.5). Two subjects were classified as heavy frame, 14 as medium, and four as small (8). Sixteen subjects were nonsmokers, and four were moderate smokers.

Before participation in the study, subjects were shown to be healthy by physical examination, including complete blood and urine biochemistry. All values were within the normal range.

Any individual with peptic ulcer, psychosis, who had ever had a myocardial infarction, or who had a history of drug allergy or diabetes was automatically excluded. Subjects were instructed to take no enzyme-inducing agents for 1 month prior to and no drugs other than the required doses of tolazamide during the study.

Subjects were advised to ingest an adequate diet of ~2500 cal/day, high in carbohydrate (minimum 300 g) for 3 days before each dose of tolazamide.

Protocol—The 20 subjects were randomly assigned to four groups of five each, and the four treatments were administered according to a 4 × 4 Latin square design. Each subject received the four treatments at 1-week intervals.

At 10 pm on the day before a tolazamide dose, subjects received a snack of two cupcakes and 240 ml (8 oz) of whole milk. Following an overnight fast, subjects reported to the clinic at 6:45 am. At 7 am 500 mg of tolazamide was administered with 180 ml of water. No food or further water were permitted until 5 hr postdose when a standard meal, consisting of one cheese sandwich, one packet of potato chips, and a small carton of milk, was provided. Food and liquid intake was unrestricted after that time.

Blood samples (10 ml) were taken from a forearm vein into evacuated glass tubes[8] (containing no anticoagulant) immediately before and then at 1, 2, 3, 4, 5, 6, 8, 12, 16, and 24 hr following tolazamide administration. Blood was allowed to clot and serum was separated by centrifugation. Serum was divided into two portions. One portion was assayed for glucose by utilizing the potassium ferricyanide–ferrocyanide oxidation reduction reaction[9] (9). The other portion was assayed for tolazamide by the high-pressure liquid chromatographic (HPLC) method described below. All samples were stored at −20° until assayed. Assays were done within 4 weeks of sampling. Assay of selected serum samples immediately after sampling and during storage showed that both tolazamide and glucose were stable at −20° for 4 weeks.

Side Effects—Subjects were monitored for drug-related side effects following each dose of tolazamide. Subjects were requested to classify each side effect as mild, moderate, or severe. Classification was subjective and no attempt was made to assess any effect quantitatively.

Assay for Tolazamide in Serum—Concentrations of tolazamide in serum were determined by an HPLC procedure developed in this laboratory. To 0.5 ml of serum was added 0.5 ml of internal standard, 5-(*p*-methylphenyl)-5-phenylhydantoin[10] in chloroform[11], 0.5 ml of aqueous sodium acetate buffer (pH 4.5), and 5 ml of methylene chloride[11]. After shaking for 5 min on a horizontal shaker and centrifuging at 3000×*g* for 3 min, the aqueous layer was discarded and the organic layer was transferred to a clean tube and evaporated to dryness under nitrogen at room temperature. The residue was reconstituted in 80 μl of methanol by vortexing, and 20 μl was injected onto the chromatograph.

The HPLC system consisted of a solvent pump[12], a fixed-volume (20 μl) sample injection valve[13], a 10-μm particle size reversed-phase octadecyl column[14] (25 cm × 4.6 mm), and a 254-nm fixed wavelength detector[15]. All chromatograms were recorded at a chart speed of 20 cm/hr.

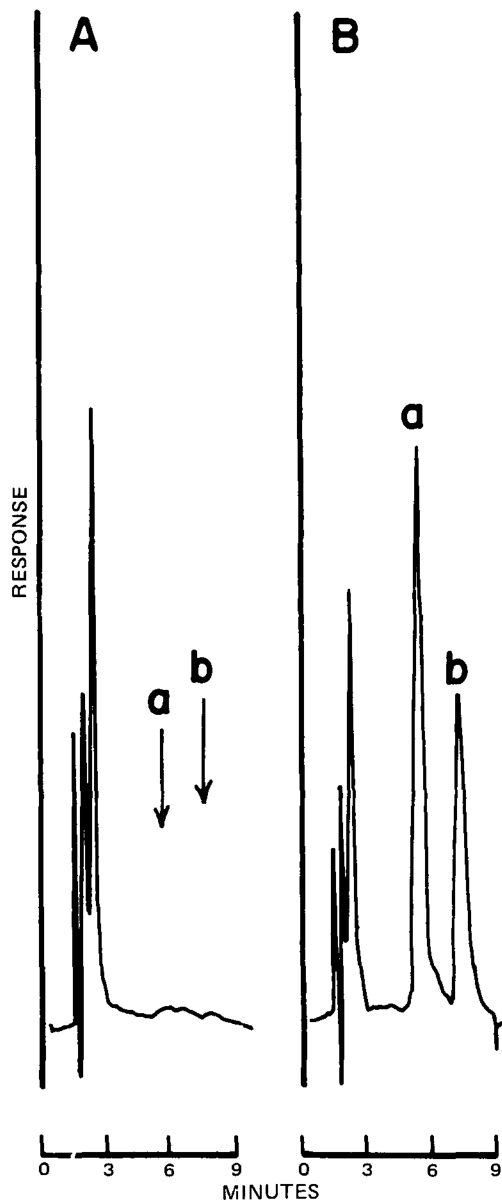

Figure 1—*Chromatograms obtained from HPLC analysis of serum containing (A) no added compounds and (B) 25 μg/ml tolazamide (a) and internal standard (b).*

The mobile phase was 52.3% methanol in pH 5.6 acetate buffer. The flow rate was 2 ml/min at a pump pressure of ~1000 psi. Concentrations of tolazamide were determined by the method of peak height ratios. Tolazamide[16] for assay standardization was reference standard quality.

Analysis of the Data—Individual serum tolazamide profiles from Formulation A were analyzed according to a single-compartment kinetic model with first-order drug appearance and elimination and an absorption lag time (10). Nonlinear regression analysis of the data was done using the program NONLIN (11) on a digital computer[17].

Estimates of the cumulative relative fraction of drug absorbed (*CRFA*) during 16 hr following each tolazamide dose were calculated by means of Eq. 1, which was derived from a previously described method for generating percent absorbed *versus* time plots (12):

$$CRFA = \frac{C_t + k_{el}^A (AUC)_{0-t}}{k_{el}^A (AUC)_{0-\infty}^A} \quad \text{(Eq. 1)}$$

[8] Vacutainer, Becton-Dickinson, Rutherford, N.J.
[9] Technicon Auto Analyzer method No. N-2b, Glucose, Technicon Instruments, Tarrytown, N.Y.
[10] Analytical grade, Aldrich Chemical Co., Milwaukee, Wis.
[11] Analytical grade, Burdick and Jackson, Muskegon, Mich.
[12] Model 110, Altex Scientific, Berkeley, Calif.
[13] Model 210, Altex Scientific, Berkeley, Calif.
[14] Lichrosorb C-18, Altex Scientific, Berkeley, Calif.
[15] Model 153, Altex Scientific, Berkeley, Calif.
[16] The Upjohn Co., Kalamazoo, Mich.
[17] IBM System/3033, IBM, White Plains, N.Y.

Table II—Mean Tolazamide Concentrations[a] in Serum

Time, hr	Treatment, μg/ml A	B	C	D	Statistic[b]
0	10.8 ± 7.4	1.3 ± 1.4	1.8 ± 1.9	3.5 ± 2.6	A D C B
1	20.5 ± 7.3	2.8 ± 2.8	5.4 ± 4.8	13.5 ± 6.6	A D C B
3	23.9 ± 5.3	4.4 ± 4.3	9.8 ± 5.6	20.0 ± 6.4	A D C B
4	25.4 ± 5.2	5.7 ± 4.1	13.6 ± 5.3	22.0 ± 5.4	A D C B
5	24.1 ± 6.3	6.6 ± 4.0	15.1 ± 4.7	22.6 ± 5.0	A D C B
6	19.9 ± 5.9	6.8 ± 3.4	14.3 ± 3.9	19.7 ± 4.7	A D C B
8	15.2 ± 5.5	6.6 ± 3.2	12.8 ± 4.1	14.6 ± 4.2	A D C B
12	8.8 ± 4.8	5.5 ± 3.2	9.1 ± 4.0	8.5 ± 4.1	C A D B
16	5.6 ± 3.8	4.6 ± 3.3	6.4 ± 3.9	5.4 ± 3.1	C A D B
24	2.7 ± 2.4	3.1 ± 2.6	3.1 ± 3.3	2.4 ± 1.8	C B A D
C_{max}[c], μg/ml	27.8 ± 5.3	7.7 ± 4.1	16.4 ± 4.4	24.0 ± 4.5	A D C B
T_{max}[d], hr	3.3 ± 0.9	7.0 ± 2.2	5.4 ± 2.0	4.0 ± 0.9	B C D A
AUC_{0-24}[e], μg hr/ml	260 ± 81	112 ± 63	193 ± 70	231 ± 67	A D C B

[a] Concentrations ±1 SD, n = 20. [b] For explanation see text. [c] Maximum concentration of tolazamide in serum. [d] Time of maximum concentration. [e] Area under the 0–24-hr serum tolazamide concentration curve calculated by trapezoidal rule.

where

$$(AUC)^A_{0-\infty} = (AUC)^A_{0-T} + \frac{C_T}{k^A_{el}},$$

C_t is the concentration of tolazamide in serum at time t, $(AUC)_{0-t}$ is the area under the tolazamide concentration *versus* time curve to time t calculated by trapezoidal rule, and C_T is the tolazamide concentration in serum at the last sampling time T. The values $k_{el}{}^A$, $(AUC)_{0-T}{}^A$, and $(AUC)_{0-\infty}{}^A$ are the first-order rate constants for loss of tolazamide from serum, the area under the tolazamide concentration curve to time T, and the area to infinite time, respectively, following Formulation A. This method permits the cumulative absorption of drug from each treatment to be calculated in individual subjects, relative to that from Formulation A.

Serum tolazamide and glucose levels from the four treatments were examined by analysis of variance for crossover design. When significant treatment effects were obtained, differences between individual products were examined by Tukey's significant difference test (13). In the statistics columns of Tables II and IV, the various values obtained from the different treatments are given in descending order of magnitude. All values under a common bar are statistically indistinguishable ($p > 0.05$); whereas, all values not under a common bar are statistically different ($p \leq 0.05$).

RESULTS

***In Vitro* Studies**—The disintegration times and dissolution rates of the four tablets are shown in Table I. While Formulations A, B, and C disintegrated within 4 min, Formulation D disintegrated more slowly with a mean time of 26.5 min. In the dissolution system used, Formulation A dissolved completely within 30 min. Formulations C and D exhibited similar dissolution characteristics to each other, with both tablets dissolving ~30%. Formulation B dissolved slowly, releasing only 10.9% of the drug during the 30-min period.

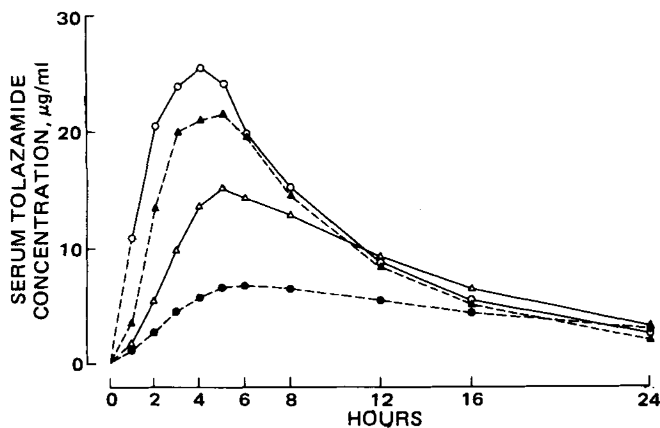

Figure 2—*Mean serum tolazamide levels as a function of time. Key: (○) Treatment A; (●) Treatment B; (△) Treatment C; (▲) Treatment D.*

Table III—Tolazamide Pharmacokinetic Parameter Values[a]

Parameter	Value
k_a[b], hr^{-1}	1.2 ± 1.3
$t_{1/2abs}$[c], hr	1.0 ± 0.7
k_{el}[d], hr^{-1}	0.18 ± 0.08
$t_{1/2elim}$[e], hr	4.6 ± 1.9
t_0[f], hr	0.75 ± 0.49
FD/V[g], μg/ml	43 ± 14
r^2[h]	0.99 ± 0.02

[a] Values ±1 SD, n = 20. [b] First-order rate constant for absorption of tolazamide in serum. [c] Absorption half-life (ln $2/k_a$). [d] First-order rate constant for loss of tolazamide from serum. [e] Elimination half-life (ln $2/k_{el}$). [f] Lag time between dosing and appearance of drug in serum. [g] Fraction of dose absorbed expressed as a concentration in the body distribution volume. [h] Coefficient of determination from nonlinear regression analysis [$r^2 = (\Sigma obs^2 - \Sigma dev^2)/\Sigma obs^2$].

Assay for Tolazamide in Serum—Typical chromatograms from serum containing both tolazamide and internal standard and from serum containing neither compound are shown in Fig. 1. Retention times for tolazamide and internal standard were 5.5 and 7.5 min, respectively. The procedure was highly specific and reproducible. Chromatographic response was linear for serum tolazamide concentrations between 1 and 100 μg/ml, and assay reproducibility from multiple replicates ($n \simeq 20$) done throughout the analytical procedures was within ±3% SD at the highest concentration and within ±12% at the lowest.

Drug-Related Side Effects—Side effects were noted following 41 of the 80 tolazamide doses. In each case the effect was judged by the subject as mild. On no occasion was supplementary medication or glucose considered to be necessary by the attending physician.

Side-effects were noted primarily 1–4 hr postdose and were generally typical manifestations of transient, mild hypoglycemia. In all there were 21 reports of lightheadedness or dizziness, 14 of excess perspiration, 12 of headache, 12 of tremor, 7 of weakness, 2 of dry mouth, 2 of drowsiness, 1 of constipation, and 1 of blurred vision.

The number of subjects reporting side effects was independent of the

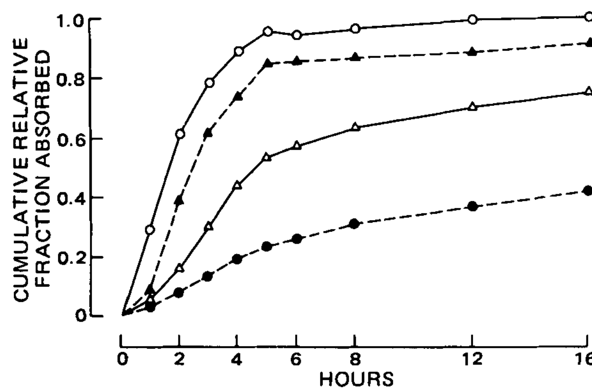

Figure 3—*Mean cumulative relative fractions of tolazamide absorbed as a function of time. Key: (○) Treatment A; (●) Treatment B; (△) Treatment C; (▲) Treatment D.*

Table IV—Parameter Values[a] for Glucose Response Following Four Tolazamide Treatments

Value	Treatment A	Treatment B	Treatment C	Treatment D	Statistic
G_{min}[b], mg/dl	54.6 ± 8.5	70.1 ± 6.2	66.0 ± 5.0	59.2 ± 6.5	B C D A
T_{min}[c], hr	1.7 ± 0.9	2.8 ± 1.0	2.6 ± 1.3	2.3 ± 1.1	B C D A
G_{dec}[d], mg/dl	41.2 ± 12.5	22.1 ± 10.2	29.2 ± 8.1	34.8 ± 6.8	A D C B
AUC_{0-5}, mg hr/dl	136 ± 41	71 ± 42	99 ± 32	116 ± 24	A D C B

[a] Values ±1 *SD*, n = 20. [b] Minimum serum glucose level. [c] Time of minimum serum glucose level. [d] Maximum decrease in serum glucose level. [e] Area under serum glucose reduction curve during 5 hr following tolazamide administration calculated by trapezoidal rule.

study phase but was dependent on treatment. Seventeen individuals reported side effects following Treatment A compared to 13 following Treatment D, 7 following Treatment C, and 4 following Treatment B.

Serum Tolazamide Levels—The mean concentrations of tolazamide in serum at each sampling time, maximum values, times of maximum values, and areas under serum tolazamide concentration curves from 0 to 24 hr, are shown in Table II. The mean data are summarized in Fig. 2.

Formulations A and D yielded serum drug profiles that were statistically indistinguishable ($p > 0.05$) at all times except 1 and 2 hr. Both treatments yielded mean peak drug levels >20 μg/ml at 3–4 hr. At 24 hr, drug levels had decreased to ~2.5 μg/ml. Serum tolazamide levels from Formulation C were lower than from A and D, the difference being significant ($p < 0.05$) at most sampling times. Formulation B yielded lower serum drug profiles than all other treatments, and the mean peak drug level of 7.7 μg/ml was not achieved from this tablet until 7 hr after drug administration.

The value of C_{max} increased in the treatment order B < C < D < A, and differences between all treatments were significant. The values of T_{max} and AUC_{0-24} were similar for Formulations A and D but were significantly different among all other pairs of treatments.

Pharmacokinetic Analysis—Analysis of individual data from Treatment A in terms of the pharmacokinetic one-compartment model yielded the values in Table III. From this product serum tolazamide levels are characterized by a mean lag time of 45 min, an absorption half-time of 1 hr, and an elimination half-life of 4.6 hr. The high coefficient of determination attests to the accuracy of the kinetic model employed. Pharmacokinetic analysis of data resulting from the other treatments is not presented, since delayed or prolonged tolazamide absorption prevented accurate determination of drug elimination parameters in some individuals receiving these products.

Estimates of *CRFA* values for each sampling time to 16 hr postdose from all four treatments are summarized in Fig. 3. The values confirm that tolazamide was absorbed at a similar rate and with similar overall efficiency from Formulations A and D. Absorption from the other two tablets was slower and less efficient, with Formulations B and C yielding *CRFA* values 42 and 75%, respectively, of Formulation A.

Serum Glucose Levels—The mean serum glucose levels during the initial 5-hr period following the four treatments are shown in Fig. 4.

The effects by the treatment on glucose levels are related to circulating drug levels. Formulations A and D caused rapid and marked reductions in serum glucose. Minimum values were observed at 1–2 hr. The reduction following Formulation C was less than from A and D and occurred at a slower rate. Formulation B had the least effect on serum glucose, and minimum levels were not achieved until 3 hr postdose.

Comparison of glucose levels at each sampling time indicates differences in glucose response between Treatments A and B and between B and D. Significant differences between Treatments A and C were observed at two sampling times and between Treatments C and D at one sampling time.

Some parameters defining the overall decrease in serum glucose are summarized in Table IV. The differences in minimum glucose levels and the maximum reduction in glucose levels were significant between all treatments. The area under the glucose response curve during the initial 5 hr following tolazamide administration was similar from Treatments A and D and from Treatments D and C but was different among all other treatment pairs. While a similar trend was observed in the 0–24-hr area, differences between treatments did not reach the 95% confidence level.

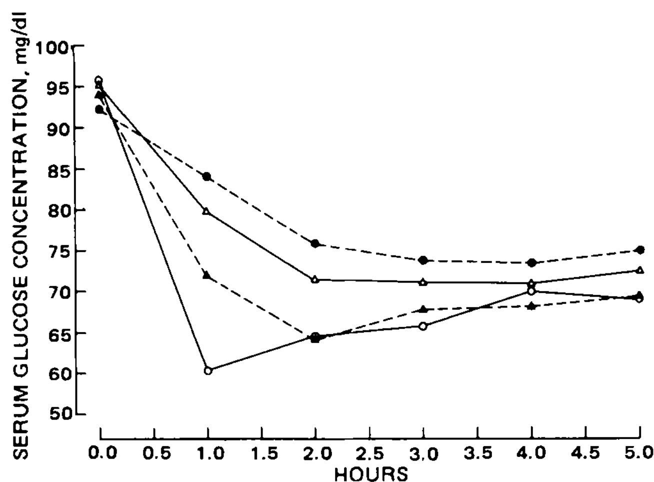

Figure 4—*Mean serum glucose concentrations as a function of time. Key: (○) Treatment A; (●) Treatment B; (△) Treatment C; (▲) Treatment D.*

DISCUSSION

Increasing interest in the use of *in vitro* disintegration and dissolution testing procedures is reflected in the proliferation of such tests in national compendia (7). The use of such tests to determine drug product bioavailability or bioequivalence has been emphasized by the U.S. Food and Drug Administration (14).

The data obtained in this study have shown that different formulations of tolazamide may give rise to marked variations in drug bioavailability and in hypoglycemic response. The ability of *in vitro* tests to predict product-related *in vivo* bioavailability changes is less clear. The official disintegration test (7) yielded similar disintegration times for Formulations A, B, and C but a more prolonged time for D. These results are at variance with the relative *in vivo* bioavailability of the four products. The dissolution test yielded rapid dissolution for Formulation A, slow dissolution for B, and intermediate values for C and D, which were indistinguishable from each other. Although this test provided a reasonable rank-order correlation with *in vivo* bioavailability, there were some important discrepancies.

Formulations C and D yielded almost identical dissolution rates to each other, which were significantly slower than A, yet Formulation D yielded serum tolazamide levels similar to those from A, and significantly higher than those from C. Thus, while the dissolution test suggests that Formulations C and D are bioequivalent to each other and that both are bioinequivalent to A, the *in vivo* results show that Formulations A and D are bioequivalent, while C is not. The two formulations giving rise to superior *in vivo* bioavailability, A and D, were prepared by wet granulation; whereas Tablets B and C were prepared by direct compression. The absolute bioavailability of tolazamide could not be determined from any treatment in this study.

Although serum glucose reduction and resulting drug-related side effects (transient, mild hypoglycemia) were less well defined between treatments than drug levels, a similar product-related trend was observed. This was particularly evident at early sampling times before food was ingested.

These results indicate that bioavailability of tolazamide from oral tablets is dissolution-rate controlled, but *in vitro* methods currently available do not predict *in vivo* drug absorption and hypoglycemic response among formulations and manufacturing processes with acceptable accuracy. For a specific formulation and manufacturing process, *in vitro* tests may be useful to assure lot-to-lot uniformity in bioavailability. However, human trials may be necessary to demonstrate that bioavailability remains reasonably constant within a given range of dissolution rates and/or disintegration times.

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Antitumor Agents LV: Effects of Genkwadaphnin and Yuanhuacine on Nucleic Acid Synthesis of P-388 Lymphocytic Leukemia Cells

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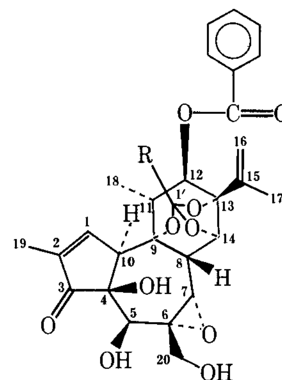
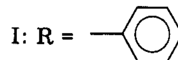
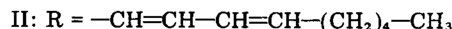
Abstract □ The diterpene esters, genkwadaphnin and yuanhuacine, have been shown to possess significant antileukemic activity in the P-388 screen. The major metabolic effects of the diterpene esters were on DNA and protein synthesis. The effects on DNA synthesis *in vitro* were evoked at a lower concentration than that required for protein synthesis inhibition. The sites in DNA synthesis which were inhibited were DNA polymerase and purine synthesis. In the latter pathway the enzyme activities inhibited were phosphoribosyl aminotransferase, inosinic acid dehydrogenase, and dihydrofolate reductase. *In vivo* administration of the diterpene esters at 0.8 mg/kg afforded identical types of effects on purine and DNA synthesis and in addition suppressed histone phosphorylation and reduced the number of surviving tumor cells. The *in vivo* effects on purine and DNA synthesis were evident as early as 6 and 24 hr after administration of a single dose of the diterpene esters.

Keyphrases □ Antitumor agents—nucleic acid synthesis by genkwadaphnin and yuanhuacine of P-388 lymphocytic leukemia cells, diterpene esters □ Genkwadaphnin—antitumor agents, nucleic acid synthesis by yuanhuacine of P-388 lymphocytic leukemia cells, diterpene esters □ Yuanhuacine—antitumor agents, nucleic acid synthesis by genkwadaphnin of P-388 lymphocytic leukemia cells, diterpene esters

Genkwadaphnin (I) and yuanhuacine (II) are two *ortho* esters bearing daphnane type diterpenes which possess an isopropylene side chain at C_{13}. Compounds belonging to the daphnane diterpene esters have previously been shown to have antileukemic activity as opposed to tigliane diterpene esters, *e.g.*, phorbol esters, which are known to be carcinogenic promoting agents (1). Compound II (odoracin or gnidilatidin) has previously been isolated from *Daphne genkwa*, *Daphne odorata*, *Gnidia latifolia*, and *Gnidia glaucus* Fres, and like other diterpene esters, such as 12-hydroxydaphnetoxin, gnidimacrin, gnidimacrin-20-palmitate, gnidilatidin-20-palmitate, gnididin, gniditrin, and gnidicin, has been demonstrated to have antileukemic activity in the 20- to 100-μg/kg dose range in rodents (2–6). The isolation and chemical characterization of a new di-







terpene ester (I) has recently been reported (7) which also has demonstrated antileukemic activity. The effects of daphnane diterpene esters on nucleic acid and protein synthesis in P-388 lymphocytic leukemia is now reported to establish a mode of action in P-388 lymphocytic leukemia cells to explain their *in vivo* antileukemic activity.

EXPERIMENTAL

The air-dried flowers of *Daphne genkwa*[1] (9 kg) (known as Yuán-Huā in Chinese folklore; Thymelaeaceae) were extracted with methanol. Guided by the *in vivo* P-388 lymphocytic leukemia rodent screen as conducted by the NCI protocol (6), the resulting active residue was dis-

[1] The plant material utilized in this investigation was identified as *Daphne genkwa* Sieb and Zucc (Thymelaeaceae) by H. C. Huang (7). A voucher sample (No. HCH-DG-771022) representing material collected for this investigation is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

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