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ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1969 from the *Department of Pharmacology, Schools of Medicine and Dentistry, Georgetown University, Washington, DC 20007*

Accepted for publication July 17, 1969.

This work was supported in part by a Hoffmann-LaRoche Fellowship.

Abstracted in part from a thesis presented by Robert E. Osterberg to the Graduate School, Georgetown University, Department of Pharmacology, in partial fulfillment of Master of Science degree requirements.

The authors wish to acknowledge the invaluable suggestions and criticisms of Drs. L. Isaac, T. E. Lynes, G. D. Maengwyn-Davies, and F. G. Standaert.

Mutual Inhibitory Effect of (−)-Mandelic Acid and Certain Sulfonamides on the Kinetics of Their Urinary Excretion in Humans

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Abstract □ Pseudo-first-order rate constants for the overall elimination of (−)-mandelic acid both in the absence (*K*) and in the presence (*Kas*) of sulfadiazine, sulfamethazine, or sulfamerazine were determined in three human subjects. Since the extent of metabolism of (−)-mandelic acid was not significantly altered in the presence of these sulfonamides, the ratio of the rate constants, *K*/*Kas*, has been calculated as a measure of the inhibitory effect of each sulfonamide on the urinary excretion of (−)-mandelic acid. For the dosage levels of the inhibitors employed, the range of such ratios found in all three subjects is 1.29–1.55 due to sulfadiazine, 1.33–1.62 due to sulfamethazine, and 1.33–1.76 due to sulfamerazine. Similar inhibitory effects were observed on the urinary excretion of sulfadiazine in the presence of (−)-mandelic acid in two subjects. Therefore, it is concluded that these compounds probably share the same renal tubular transport system(s) for their secretion in humans.

Keyphrases □ (−)-Mandelic acid urinary excretion—sulfonamide effect □ Sulfonamide urinary excretion—(−)-mandelic acid effect □ Urinary excretion, sulfonamides, (−)-mandelic acid—mutual inhibition □ GLC—analysis □ UV spectrophotometry—analysis

Since the time Marshall *et al.* (1–3) produced evidence for the renal tubular secretion of phenol red, numerous studies have been conducted to show that other substances are also secreted by the renal tubules. In addition, considerable efforts are being made to establish the mechanism of renal tubular secretion. Weiner and Mudge (4–6) have reviewed the tubular mechanisms for the excretion of organic acids and bases while Despopoulos (7) has reviewed the renal transport of organic ions. Current theory suggests the presence of separate mechanisms for renal tubular secretion of acids and bases in humans (4). Extensive lists of drugs, which are secreted by the renal tubules, are cited by Weiner and Mudge (4) and Despopoulos (7).

Nagwekar and Kostenbauder (8) have demonstrated that the excretion and metabolism of both optical isomers of mandelic acid follow pseudo-first-order kinetics in humans and that there is no significant difference in the rate constants for excretion of these isomers. They showed that the principal metabolites of mandelic acid are benzoylformic acid and benzoic acid and that mandelic acid is completely recovered in the urine. The rate of metabolism of (+)-mandelic acid was found to be twice the rate of metabolism of (−)-mandelic acid. A gas-chromatographic method was used to quantitatively determine intact mandelic acid and its metabolites excreted in the urine.

Nagwekar and Kostenbauder (8) also studied in humans the effect of probenecid on the urinary excretion of mandelic acid and since probenecid decreased the rate of urinary excretion of both isomers of mandelic acid, they concluded that mandelic acid is probably involved in active renal tubular transport.

Although Despopoulos and Callahan (9) could show no effect of probenecid on the rate of transport of sulfadiazine, sulfamerazine, and sulfamethazine in rabbit kidney slice studies, Crosley *et al.* (10) demonstrated, in humans, an increase in the plasma levels of both the intact and the acetylated forms of triple sulfonamides in the presence of probenecid. They attributed this to the inhibitory effect of probenecid on the excretion of these sulfonamide(s). Hansen *et al.* (11) also studied the effect of probenecid on the plasma concentration of the same triple sulfonamide mixture in humans, and reported an increase in the plasma concentration of intact sulfonamide in some of their studies.

These experimental observations suggest that both sulfonamides and the mandelic acids may be secreted by an active transport mechanism present in the kidney tubules, and that probenecid interferes with this active process. They also suggest that both sulfonamides and the mandelic acids are secreted by the same mechanism. If this is the case, an optical isomer of mandelic acid and a sulfonamide, when administered simultaneously

to human subjects, should compete for the secretory mechanism and decrease the excretion rate constant of each other. The purpose of the present study was to investigate this possibility by determining the mutual inhibitory effect of (−)-mandelic acid and certain sulfonamides on the kinetics of their respective urinary excretion in humans.

EXPERIMENTAL

Materials—(−)-Mandelic acid (Aldrich Chemical Co.), recrystallized, m.p. 132–133°, $[\alpha]_D^{25}$ −154.4°; sulfadiazine (Retort Pharmaceutical Co.), m.p. 251–253°; sulfamethazine (Robinson Labs. Inc.) m.p. 175–176°; sulfamerazine (Amend Drug and Chemical Co.) m.p. 232–234°.

Apparatus—The gas chromatograph employed (F & M model 810R-19) was equipped with a hydrogen flame detector. The column used was a copper tube 1.22 m. (4 ft.) long and 0.63 cm. (0.25 in.) outside diameter packed with diatomaceous earth (Diatoport S, 80–100 mesh) coated with 5% ethylene glycol succinate. Helium was used as the carrier gas. A 10-μl. standard syringe (Hamilton) was used for injecting the samples onto the column. A spectrophotometer (Beckman model DK-2A) was used in this study. A pH meter (Beckman model 72) was used for all pH determinations.

Subjects and Test Procedures—Three apparently healthy adult male subjects (age range 25–36 years, weight range 170–210 lb.) participated in this study. Each subject, after overnight fasting, ingested 1 g. of (−)-mandelic acid. The subject was advised against eating anything for at least 2 hr. after ingesting the drug but no other dietary restrictions were imposed. Each subject collected a blank urine sample prior to ingestion of the drug. Following the ingestion of (−)-mandelic acid, urine was quantitatively collected at intervals of 1 hr. for 10 hr., and then at longer intervals up to 36 hr. The urine samples were allowed to attain room temperature, volume and pH were measured, and they were stored in the refrigerator until the time they were analyzed. No attempt was made to control the pH of urine during the excretion studies. The pH of the urine generally remained about 6.0 ± 1.0 throughout the study. Each subject ingested (−) mandelic acid in separate studies generally after an interval of 1 week.

In the studies involving a sulfonamide, the subject ingested 1 g. of (−)-mandelic acid after overnight fasting and collected urine quantitatively every hour for 4 hr. At the end of 4 hr., the subject collected the fourth urine sample and immediately ingested the appropriate sulfonamide. Volume and pH of the urine samples were recorded in the usual manner. In these studies, the subject was advised to eat only a light breakfast immediately after the collection of the urine sample 2 hr. following the ingestion of (−)-mandelic acid. The subject was further advised against eating for at least 2 hr. after the ingestion of the sulfonamide.

When the kinetics of urinary excretion of sulfadiazine were studied, the subject, after overnight fasting, ingested a 1-g. dose of sulfadiazine. Urine samples were quantitatively collected at 3-hr. intervals for the first 15 hr. and at longer intervals up to 48 hr. Volume and pH of the urine samples were recorded in the usual manner.

When the excretion of sulfadiazine was studied in the presence of (−)-mandelic acid, the subject, after overnight fasting, ingested a 1.3-g. dose of sulfadiazine and collected urine at 3-hr. intervals for the first 24 hr. and at 1 to 1.5-hr. intervals up to 28–30 hr. Immediately after collecting the urine sample at the end of this period, the subject ingested a 1-g. dose of (−)-mandelic acid followed by seven successive 0.5-g. doses of the acid at intervals of 50 min. Urine samples were quantitatively collected at 1-hr. intervals for the first 6 hr. after the initial ingestion of (−)-mandelic acid. Volume and pH of the urine samples were recorded in the usual manner.

Both (−)-mandelic acid and the sulfonamides as ingested by the subjects, were dispensed in hard gelatin capsules.

Analysis of Mandelic Acid and Its Metabolites—An appropriate volume of each urine sample, usually 1/4 of the total volume, was pipetted into a suitable separator. The urine sample was adjusted to approximately pH 2 with 5 *N* HCl and mandelic acid and its metabolites were completely extracted with alcohol-free ether. The volume of the ether used for each of four extractions was about twice the volume of the aqueous phase. Each of the ether extracts was transferred to a suitable beaker and the ether evaporated on a water bath at about 55°. The residue was dissolved in approximately a 2-ml. quantity of ether and quantitatively transferred to a 15-ml. graduated tube using additional ether. The ether was then evaporated and a sufficient quantity of ethereal diazomethane was added to the tube to convert the carboxylic acids, which were primarily intact mandelic acid and its metabolites, to their respective methyl esters. The ether and excess diazomethane were evaporated on a water bath at 55° and a sufficient quantity of methanol was added to the tube to dissolve the residue and yield the desired concentration of 5–15 mg. mandelic acid/2 ml. of the solution. Blank urine was also treated in exactly the same manner as the urine samples. The methanolic solutions were then analyzed gas chromatographically. A typical chromatogram obtained in these studies is shown in Fig. 1.

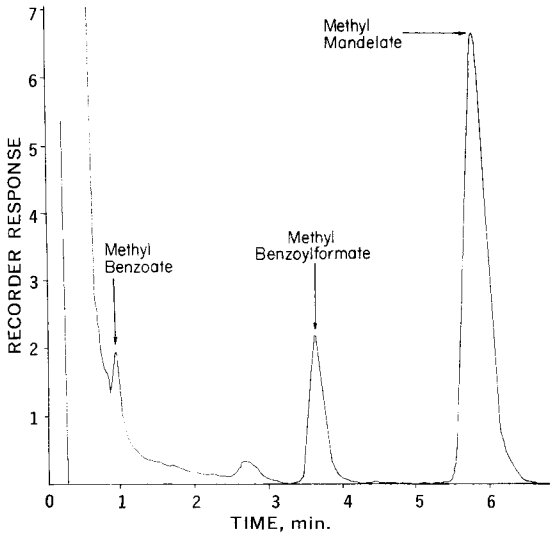


Figure 1—*A typical gas chromatogram, obtained for 7th-hr. urine sample of one of the subjects, shows distinct peaks for methyl esters of mandelic acid and its metabolites. Conditions: range, 10; attenuation, 64×: injection port temperature, 210°; column temperature, 165°; detector temperature, 220°; helium flow, 60 ml./min.*

In order to accurately determine the amount of mandelic acid and its metabolites present in the urine samples, calibration curves of concentration *versus* area under the chromatographic curve for the respective acids were constructed each time the urine samples were analyzed by gas chromatographic methods. For the construction of calibration curves appropriate volumes of aqueous solutions containing known quantities of these acids were treated in the same manner as the urine samples to yield the methyl esters.

Areas under the chromatographic curve were calculated by the trapezoidal rule (12) and the quantity of each ester, methyl mandelate, methyl benzoylformate, and methyl benzoate, was determined.

Analysis of Sulfadiazine—The procedure used for the analysis of sulfadiazine and *N*-4-acetylsulfadiazine present in the urine samples was that of Bratton and Marshall (13). In order to determine the amount of acetylated sulfadiazine excreted in the urine, all the urine samples of a study were combined and the appropriate volume was acidified with 0.1 *N* HCl and heated in a water bath at 100° for 2 hr. The sulfadiazine liberated upon the hydrolysis of *N*-2-acetylsulfadiazine, and that present initially in the sample, was determined in the manner described above.

RESULTS AND DISCUSSION

Selection of Agents—In order to study the inhibitory effect of one compound on the urinary excretion of another, it is desirable, whenever possible, to employ compounds which undergo minimal metabolism. The purpose of this study was not only to determine the mutual inhibitory effect of (−)-mandelic acid and certain sulfonamides on the kinetics of their urinary excretion, but also to attempt to study the effect of systematic variation of a hydrophobic group, such as methyl groups, in a sulfonamide molecule

Table I—Summary of Urinary Excretion Recovery Data Obtained Following Oral Administration of (−)-Mandelic Acid[a] and the Appropriate Sulfonamide to Three Male Human Subjects

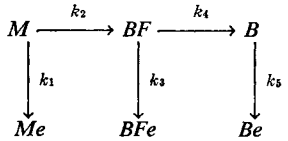
Subject	Sulfonamide Administered	Mandelic Acid Excreted as— Intact Mandelic Acid, mg.	Benzoylformic Acid, mg.	Benzoic Acid, mg.	% Metabolized[b]	Total Mandelic Acid Excreted, mg.
A	None	763	169	20	19.9	952
A	Sulfadiazine	833	129	13	14.6	975
A	Sulfamethazine	792	148	16	17.2	956
A	Sulfamerazine	677	197	1	22.6	875
B	None	923	108	18	12.0	1049
B	Sulfadiazine	737	68	33	12.1	838
B	Sulfamethazine	806	83	17	11.0	906
B	Sulfamerazine	876	130	4	13.3	1010
C	None	927	116	23	13.0	1066
C	Sulfadiazine-1[c]	894	93	5	9.9	992
C	Sulfamethazine-1	847	104	3	11.2	954
C	Sulfamerazine-1	799	137	2	14.8	938
C	Sulfadiazine-2[c]	831	104	4	11.5	939
C	Sulfamethazine-2	846	77	15	9.8	938
C	Sulfamerazine-2	789	82	8	10.2	879

[a] The dose of (−)-mandelic acid ranged from 992 to 1018 mg. [b] The percentage is based on the total amount of mandelic acid recovered in the urine. [c] These numbers represent Studies 1 and 2 in Subject *C*.

on the urinary excretion of mandelic acid. Therefore, sulfadiazine, sulfamethazine, and sulfamerazine, the members of a homologous series, were used in this study. Although these sulfonamides are metabolized to a significant extent, they are employed in this study since they are used therapeutically and represent a homologous series which provides a desirable systematic variation in the hydrophobic group. The (−)-mandelic acid was chosen in this study since it is metabolized only to the extent of 10% of the administered dose in humans (8).

Ethanol-free ether was used in all phases of the analytical procedure. When commercial ether, which contains about 3% ethanol, was employed for the preparation of the urine samples for gas chromatographic analysis, the chromatograms obtained exhibited extraneous peaks which interfered with the quantitative determination of methyl mandelate and methyl benzoylformate. The use of commercial ether, which was distilled following overnight refluxing with sodium metal for the removal of ethanol, eliminated the appearance of the extraneous peaks and allowed the quantitative determination of the methyl esters of mandelic acid and benzoylformic acid. Since Huyser and Neckers (14) have shown that a reaction occurs between esters of benzoylformic acid, such as the ethyl ester, and certain alcohols, such as 2-butanol, cyclohexanol, and menthol, it seemed possible that ethyl benzoylformate was produced by a similar reaction between the methyl ester of benzoylformic acid and the ethanol present in the ether used in the extraction procedure as well as in the ethereal solution of diazomethane. Ethyl benzoylformate was prepared by the standard procedure (15) by esterifying benzoylformic acid with ethanol in the presence of concentrated sulfuric acid. Since the retention times observed for one of the extraneous peaks after treating benzoylformic acid with untreated commercial ether was identical to the retention time observed for the ester prepared by the above method, such an extraneous peak was attributed to the ethyl ester of benzoylformic acid. However, no further attempts were made to identify the chemical entities causing the extraneous peaks.

Kinetics of Urinary Excretion of (−)-Mandelic Acid—Prior to the study of the inhibitory effect of the sulfonamides on the kinetics of urinary excretion of (−)-mandelic acid, it was necessary to determine the rate constant of elimination of (−)-mandelic acid in each of the subjects of this study. The first-order kinetic model, which has been described by Nagwekar and Kostenbauder (8) for the elimination of mandelic acid in humans, may be shown as follows.



Model I

where M is the amount of mandelic acid in the body at any time; BF is the amount of benzoylformic acid in the body at any time; B is the amount of benzoic acid in the body at any time; *Me*, *BFe*, and *Be* are the amounts of the respective acids excreted in the urine at any time; and k_i ($i = 1 \ldots 5$) represent apparent first-order rate constants with dimensions of reciprocal time for the respective processes indicated in the above model.

Since the main purpose was to determine the rate constant of elimination of (−)-mandelic acid from the urinary excretion data, appropriate differential equations for the above model are described below.

$$\frac{dM}{dt} = -(k_1 + k_2)M = -KM \qquad \text{(Eq. 1)}$$

where $K = k_1 + k_2$

$$\frac{dMe}{dt} = k_1 M \qquad \text{(Eq. 2)}$$

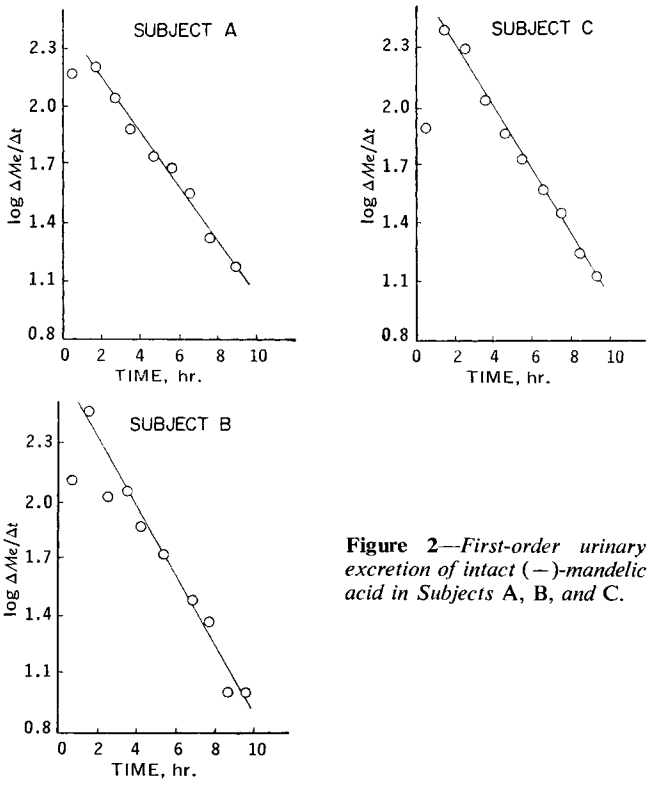


Figure 2—*First-order urinary excretion of intact (−)-mandelic acid in Subjects* A, B, *and* C.

Upon integration and evaluation of the constant of integration and then expressing the equation in the exponential form, Eq. 1 becomes

$$M = M_0 e^{-Kt} \qquad \text{(Eq. 3)}$$

where M_0 is the hypothetical amount of mandelic acid in the body at zero time and K is the overall rate constant for the elimination of mandelic acid from the body.

After substituting Eq. 3, Eq. 2 may be written as

$$\frac{\Delta Me}{\Delta t} = k_1 M_0 e^{-Kt}$$

or

$$\log \frac{\Delta Me}{\Delta t} = \log k_1 M_0 - Kt/2.303 \qquad \text{(Eq. 4)}$$

where $\Delta Me/\Delta t$ is the amount of intact mandelic acid excreted in the urine in unit time. The log $\Delta Me/\Delta t$ is plotted against t and from the slope of the resulting straight line, the rate constant for overall elimination of (−)-mandelic acid is calculated. The straight-line plot is obtained by the method of least squares after a postabsorptive and postequilibrative period. The urinary excretion recovery data obtained for each subject following the ingestion of (−)-mandelic acid are presented in Table I and the plots of log $\Delta Me/\Delta t$ *versus* t are shown in Fig. 2. The time t in these and other figures represent the midpoints of the urinary collection intervals (16).

The rate constants for overall elimination of (−)-mandelic acid observed for Subjects *B* and *C* are identical, 0.376 hr.$^{-1}$, and that observed for Subject *A* is 0.308 hr.$^{-1}$. The range of rate constants obtained in these studies is comparable to that obtained previously (8) upon similar treatment of the data.

Inhibitory Effect of Sulfonamides on the Urinary Excretion of (−)-Mandelic Acid—The inhibitory effect of sulfadiazine, sulfamethazine, and sulfamerazine on the urinary excretion of intact (−)-mandelic acid has been observed in all three subjects and data for one of the subjects are presented in Fig. 3. The rate constant, K, for overall elimination of (−)-mandelic acid was calculated from the slope of the straight line obtained after a postabsorptive and postequilibrative period of the acid prior to the administration of the appropriate sulfonamide. A modified overall rate constant of elimination, *Kas*, was calculated from the straight line obtained after 1.5 hr. following the oral administration of the appropriate sulfonamide. These overall elimination rate constants are listed in Table II. The period of 1.5 hr. was allowed for substantial absorption of the sulfonamide.

Table II—Rate Constants for Overall Elimination of (−)-Mandelic Acid Both in the Absence and in the Presence of Respective Sulfonamides and the Index of Inhibitory Effect, *R*, of Each Sulfonamide on the Urinary Excretion of (−)-Mandelic Acid in Three Male Human Subjects

Subject	Sulfonamide	K(hr.$^{-1}$)	*Kas*(hr.$^{-1}$)	R^a
A	Sulfadiazine	0.436	0.282	1.55
A	Sulfamethazine	0.495	0.371	1.33
A	Sulfamerazine	0.463	0.263	1.76
B	Sulfadiazine	0.460	0.325	1.42
B	Sulfamethazine	0.458	0.301	1.52
B	Sulfamerazine	0.447	0.317	1.41
C	Sulfadiazine-1	0.531	0.400	1.33
C	Sulfadiazine-2	0.773	0.597	1.29
C	Sulfamethazine-1[b]	0.465	0.287	1.62
C	Sulfamethazine-2[b]	0.509	0.354	1.44
C	Sulfamerazine-1	0.397	0.299	1.33
C	Sulfamerazine-2	0.542	0.394	1.38

[a] In order to emphasize the significance of these values, the rate constants for overall elimination of (−)-mandelic acid in the absence of a sulfonamide during the period of 2–4 hr. and 6–9 hr. (Fig. 2) were determined and the "*R*" values calculated. These *R* values obtained for Subjects *A*, *B*, and *C* were found to be 1.18, 1.14, 1.17, respectively. The elimination rate constants determined during the period of 2–4 hr. for Subjects *A*, *B*, and *C* are 0.363 hr.$^{-1}$, 0.472 hr.$^{-1}$, and 0.427 hr.$^{-1}$, respectively. [b] These numbers represent studies 1 and 2 in subject C.

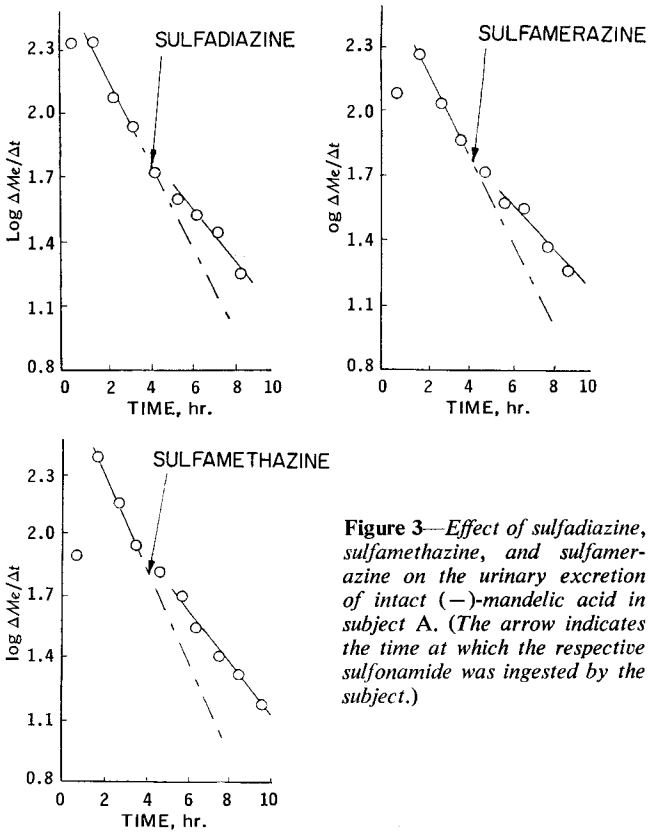


Figure 3—*Effect of sulfadiazine, sulfamethazine, and sulfamerazine on the urinary excretion of intact (−)-mandelic acid in subject* A. *(The arrow indicates the time at which the respective sulfonamide was ingested by the subject.)*

The values of *R*, which are obtained from the ratio of *K*/*Kas* to determine the inhibitory effect of each sulfonamide, are listed in Table II. In this paper, the *R* values are used as the index of the inhibitory effect. Since each of the values of *R* is greater than unity, it indicates the inhibitory effect of each sulfonamide on the urinary excretion of intact (−)-mandelic acid.

It may be observed from Table II that the rate constants, *K*, determined in all the inhibitory studies performed in Subject *B* and in the sulfamethazine-1 and sulfamerazine-1 studies performed in Subject *C* ranged from 0.397 to 0.465 hr.$^{-1}$ and that these values are not much different from those rate constants determined during a similar period of time for Subject *B* (0.472 hr.$^{-1}$) and Subject *C* (0.427 hr.$^{-1}$) in the studies involving only (−)-mandelic acid. Upon similar evaluation, the rate constant determined in the inhibitory studies in Subject *A* ranged from 0.436 to 0.495 hr.$^{-1}$ and these values are higher than the 0.363 hr.$^{-1}$ value of the rate constant determined for this subject in the study involving only (−)-mandelic acid. Such differences in the similarly evaluated rate constants are also noticed in some of the studies of Subject *C*. It may, however, be pointed out that the values of *R* determined in all the inhibitory studies involving sulfadiazine, sulfamethazine, and sulfamerazine are in the range 1.29–1.55, 1.33–1.62, and 1.33–1.76, respectively (Table II).

From these results, it appears that the extent of the inhibitory effect of these sulfonamides upon the urinary excretion rate of (−)-mandelic acid is not significantly different from each other. Furthermore, conclusions regarding the effect of systematic variation of hydrophobic groups in the sulfonamide molecule on the rate of urinary excretion of (−)-mandelic acid becomes difficult because of the significant differences in binding capacities of these sulfonamides to the plasma proteins (17), in their pKa values (7), and their extent of metabolism in the body (17).

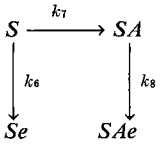
Kinetics of Urinary Excretion of Sulfadiazine—Although Sadusk and Tredway (18) observed the excretion and acetylation of sulfadiazine in man, they did not determine the rate constants of overall elimination of this drug. However, upon treating their data, which were obtained after administration of a 3-g. dose of the drug to the subject, it was observed that the urinary excretion of sulfadiazine seemed to follow a pseudo-first-order kinetic process. Since other sulfonamides, such as sulfaethylthiadiazole (19) and sulfiasoxazole (20) have been found to be excreted by a pseudo-first-order process,

Table III—Summary of Urinary Excretion Recovery Data Obtained Following Oral Administration of Sulfadiazine in the Absence and in the Presence of (−)-Mandelic Acid to Two Male Human Subjects

Subject	Mandelic Acid	Dose Administered, mg.	Sulfadiazine Excreted—Intact, mg.	Sulfadiazine Excreted—Acetylated, mg.	% Metabolized[a]	Total Sulfadiazine Excreted, mg.
B	Absent	1005	839	178	17.5	1017
B	Present	1465	1337	156	10.5	1489
C	Absent-1[b]	1005	698	206	22.8	904
C	Absent-2[b]	1000	795	196	19.8	991
C	Present-1	1339	907	397	30.4	1304
C	Present-2	1369	929	307	24.8	1236

[a] The percentage is based on the total amount of sulfadiazine recovered in the urine. [b] These numbers represent Studies 1 and 2 in Subject *C*.

it seems probable that the overall elimination of sulfadiazine also follows a pseudo-first-order process. Further, it has been shown that the major metabolic product reported for sulfadiazine is *N*-4-acetylsulfadiazine (17). Therefore, the data obtained in these studies are treated according to the following model for elimination of sulfadiazine (21):



Model II

where S is the amount of sulfadiazine in the body at any time; SA is the amount of acetylated sulfadiazine in the body at any time; Se and SAe are the respective amounts of intact sulfadiazine and *N*-4-acetylsulfadiazine excreted in the urine at any time; and k_i (i = 6, 7, 8) represent apparent first-order rate constants with dimensions of reciprocal time for the respective processes indicated in the above model.

Since the main purpose was to determine the rate constant of elimination of sulfadiazine from the body, the following equation may be written from the above model:

$$\log \frac{\Delta Se}{\Delta t} = \log k_6 S_0 - Ks(t)/2.303 \qquad \text{(Eq. 5)}$$

where S_0 is the hypothetical amount of intact sulfadiazine in the body at zero time; Ks is the overall rate constant for the disappearance of sulfadiazine from the body ($Ks = k_6 + k_7$); and $\Delta Se/\Delta t$ is the amount of intact sulfadiazine excreted in the urine in unit time.

The urinary excretion recovery data obtained for sulfadiazine are presented in Table III. The log $\Delta Se/\Delta t$ is plotted against t (Figs. 4 and 5) and, from the slope of the resulting straight line, the overall elimination rate constant for sulfadiazine, Ks, is calculated. The straight lines were obtained by the method of least squares after a postabsorptive and postequilibrative period. The rate constant for overall elimination of sulfadiazine observed for Subject *B* is 0.0755 hr.$^{-1}$ and that determined for Subject *C* in two separate studies are 0.0841 and 0.0967 hr.$^{-1}$

Inhibitory Effect of (−)-Mandelic Acid on the Urinary Excretion of Sulfadiazine—As shown in Figs. 6 and 7, the inhibitory effect of mandelic acid on the urinary excretion of intact sulfadiazine has been observed in Subjects *B* and *C*. The overall elimination rate constant, Ks, was calculated from the slope of the straight line for the data obtained after a postabsorptive and postequilibrative period prior to the administration of (−)-mandelic acid. The value of this rate constant as determined for Subject *B* is 0.0612 hr.$^{-1}$ and those determined for Subject *C* in two separate studies are 0.0822 and 0.0980 hr.$^{-1}$. These values of the rate constant are comparable to those determined in the respective subjects in the studies involving only sulfadiazine. Since repeated oral doses of (−)-mandelic acid were administered to the subjects in order to study the inhibitory effect of (−)-mandelic acid on the urinary excretion of sulfadiazine, it was not considered appropriate to calculate the first-order rate constant for overall elimination of sulfadiazine during the period of administration of (−)-mandelic acid. However, during this period, the scatter of points observed in Figs. 6 and 7 is not prohibitive to the assumption that the elimination of sulfadiazine occurred by a pseudo-first-order process and

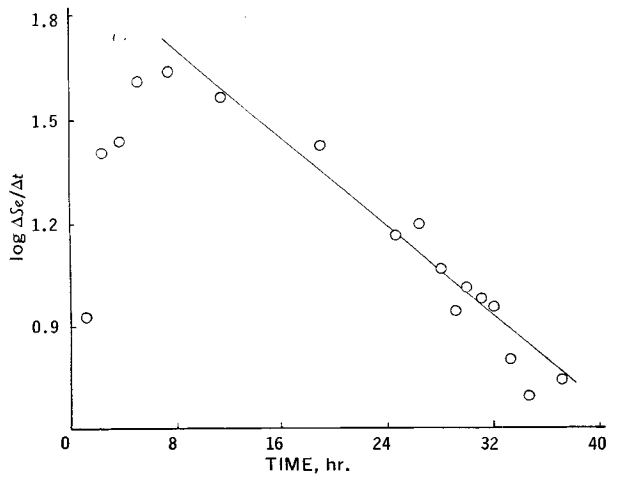


Figure 4—*First-order urinary excretion of intact sulfadiazine in Subject* B.

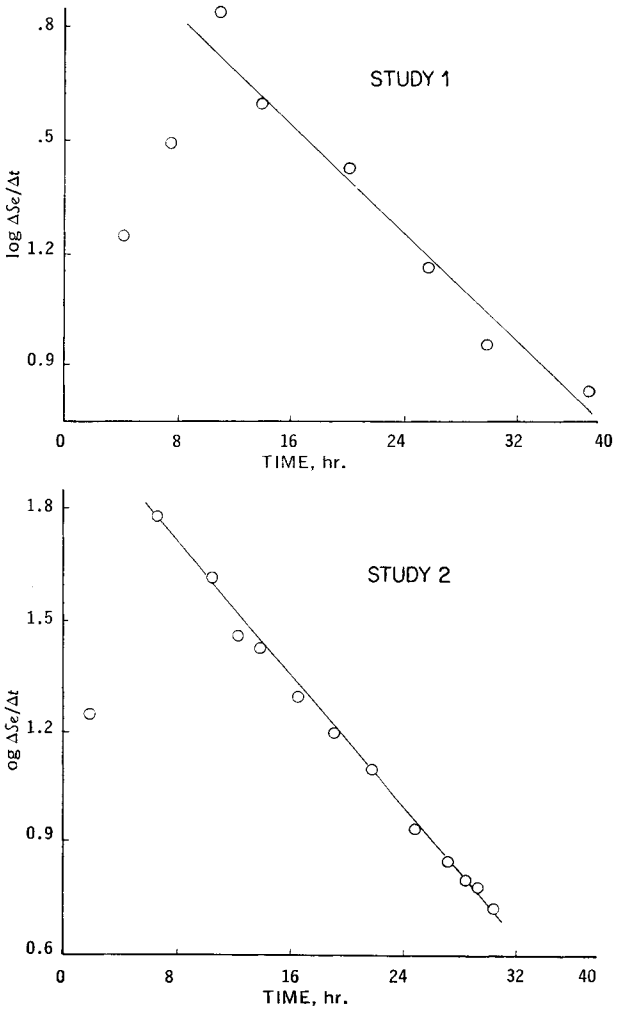


Figure 5—*First-order urinary excretion of intact sulfadiazine in Subject* C.

thereby attempt to estimate the inhibitory effect of (−)-mandelic acid in terms of *R* values. Therefore, the values of *Kam*, the rate constant of elimination of sulfadiazine during the administration of (−)-mandelic acid, were determined from the slope of the dotted straight lines obtained for the appropriate data by the method of least squares (Table IV). The value of *Rs* (*Ks*/*Kam*) obtained for Subject *B* is 2.05 and those obtained for Subject *C* in two separate studies are 1.63 and 1.77. The dotted straight lines in Figs. 6 and 7 suggest that the lines were not theoretically expected from these data.

Since it was convenient to ingest doses of (−)-mandelic acid after an elapsed period of two biological half-lives of sulfadiazine, a dose of 1.3 g. of sulfadiazine was ingested by Subjects *B* and *C*. The dose of 1.3 g. of sulfadiazine was administered to provide a substantial amount of sulfadiazine to be present in the body at the time of ingestion of (−)-mandelic acid. Since the biological half-life of (−)-mandelic acid was approximately 1/4 that of sulfadiazine, the dosage schedule followed for the repeated administration of (−)-mandelic acid was designed to provide a significantly high concentration of (−)-mandelic acid in the body during the period of the inhibitory studies.

In the studies dealing with the inhibitory effect of sulfonamides on the urinary excretion of (−)-mandelic acid, it may be pointed out that, in addition to intact sulfonamides, factors such as the binding of sulfonamides to the plasma proteins, the pKa values of the sulfonamides in relation to the urinary pH, and the possible interference of the *N*-4-acetylated sulfonamide may be believed to have played a role in modifying the excretion rate of (−)-mandelic acid, but the contribution of each of these factors could not be determined under the conditions of this study. Such factors might also be believed to have played a role in modifying the urinary excretion rate of sulfadiazine in the presence of (−)-mandelic acid. The effect of pH on the urinary excretion and tubular reabsorption of drugs has been discussed and demonstrated by several workers (5, 6). The urine pH of all the subjects throughout this study was generally found to be in the pH range of 5.0–7.0. Considering the pKa values of the agents employed in this study (mandelic acid, pKa 3.9; sulfadiazine, pKa 6.5; sulfamethazine, pKa 7.4; sulfamerazine, pKa 7.1), although the rate of excretion of sulfonamides may be expected to be affected, it does not seem possible that the rate of urinary excretion of mandelic acid would be affected in this pH range. It may further be pointed out that the examination of Fig. 5 (Study 2) shows no apparent effect of pH on the kinetics of urinary excretion of sulfadiazine, even though the urine pH in that study ranged from 4.85 to 6.90.

It is further noticed that in most of the present studies (Figs. 3, 7), the rate of excretion of the agent under study slightly increased instead of decreasing, immediately following the administration of the inhibitor. Since it was thought that this increase in the excretion rate might be due to an increase in the plasma concentration of the free form of the compound as a result of its displacement by the inhibitor from the protein-binding site, *in vitro* binding studies (22) were undertaken. These studies showed that both (−)-mandelic acid and sulfadiazine were able to decrease the extent of their protein binding in the presence of each other. Therefore, the increase in the excretion rate referred to above is attributed to the possible displacement of protein-bound drug.

Although the term overall elimination rate constant is comprised of both the excretion rate constant and the metabolic rate constant, the ratios *K*/*Kas* in the present studies are considered as the indexes of inhibition of urinary excretion of (−)-mandelic acid. The rationale for this becomes evident from the examination of data presented in Table I. According to these data, no apparent change in the extent of metabolism of (−)-mandelic acid in the presence of sulfadiazine, sulfamethazine, or sulfamerazine was observed, indicating that the rate constant for metabolism of (−)-mandelic acid remains

Table IV—Rate Constants for Overall Elimination of Sulfadiazine Both in the Absence and in the Presence of (−)-Mandelic Acid and the Index of Inhibitory Effect, *Rs*, of (−)-Mandelic Acid on the Urinary Excretion of Sulfadiazine in Two Male Human Subjects

Subject	*Ks*(hr.$^{-1}$)	*Kam*(hr.$^{-1}$)[a]	*Rs*[a]
B	0.0612	0.0299	2.05
C-1[b]	0.0822	0.0504	1.63
C-2[b]	0.0980	0.0554	1.77

[a] The basis for estimating the values of *Kam* and *Rs* is explained in the text. [b] The numbers represent Studies 1 and 2 in Subject *C*.

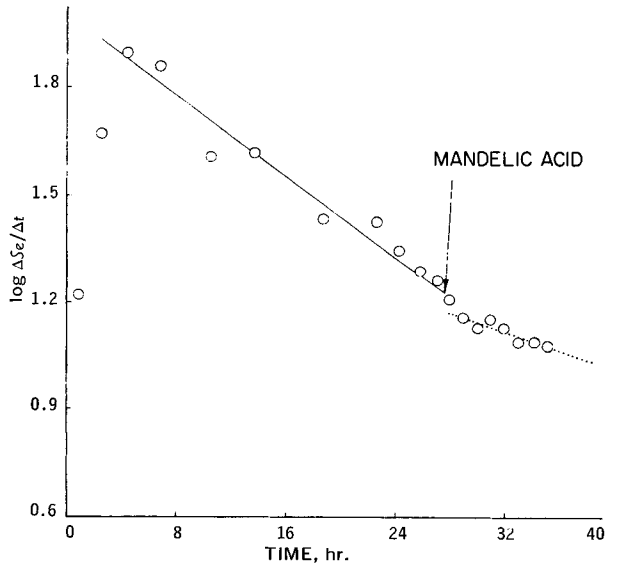


Figure 6 —*Effect of* (−)-*mandelic acid on the urinary excretion of intact sulfadiazine in Subject* B. [*The arrow indicates the time at which the initial dose of* (−)-*mandelic acid was ingested by the subject. The reason for the dotted line is explained in the text.*]

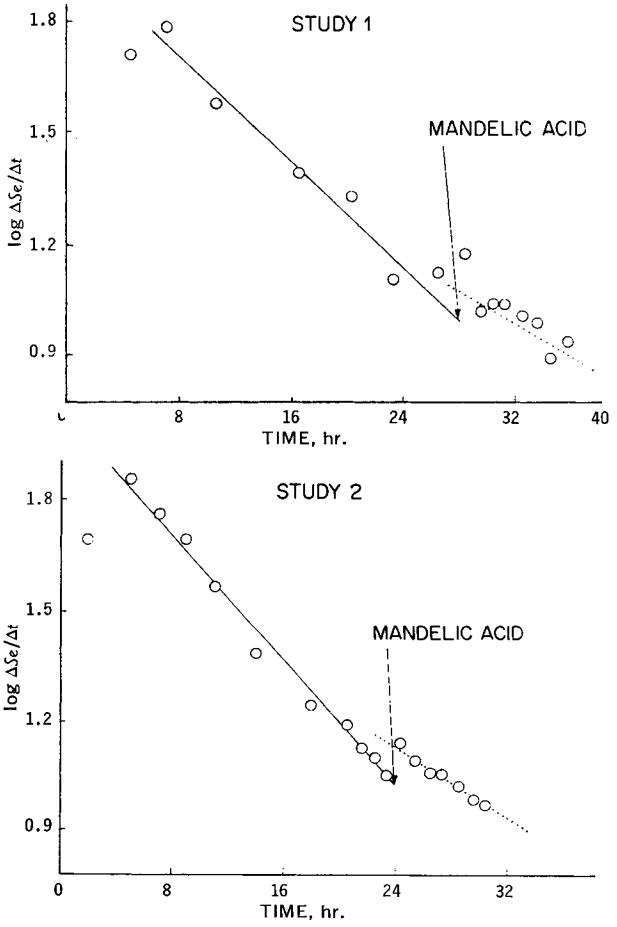


Figure 7 – *Effect of* (−)-*mandelic acid on the urinary excretion of intact sulfadiazine in Subject* C. [*The arrow indicates the time at which the initial dose of* (−)-*mandelic acid was ingested by the subject. The reason for the dotted line is explained in the text.*]

practically constant in the presence of these sulfonamides. Therefore the changes observed in the values of the overall elimination rate constant of (−)-mandelic acid are attributed to the changes in the secretion rate of mandelic acid in the presence of each of the sulfonamides. Similarly, upon examination of the data for sulfadiazine (Table III), it may be concluded that the changes observed in the overall elimination of sulfadiazine are attributed to the changes in the secretion rate of sulfadiazine in the presence of mandelic acid.

The inhibitory effect of (−)-mandelic acid observed on the urinary excretion of sulfadiazine has provided evidence that intact sulfadiazine, or for that matter intact sulfonamide, is able to inhibit the elimination rate of (−)-mandelic acid. These studies indicate the mutual inhibitory effect of (−)mandelic acid and the sulfonamides (sulfadiazine, sulfamethazine, and sulfamerazine) on the kinetics of their urinary excretion in humans. Although the determination of the effect of structural variation in the sulfonamide molecules of this study on the urinary excretion of (−)-mandelic acid is complicated by the various factors mentioned previously, this study demonstrates that (−)-mandelic acid and these sulfonamides are probably transported by the same mechanism present in the kidney tubules.

SUMMARY AND CONCLUSIONS

Studies were carried out to determine the mutual inhibitory effect of (−)-mandelic acid and certain sulfonamides on the kinetics of their urinary excretion in adult male human subjects.

The rate constants for the overall elimination of (−)-mandelic acid in the absence of a sulfonamide (*K*) and in the presence of sulfadiazine, sulfamethazine, or sulfamerazine (*Kas*) were determined.

The ratios of *K*/*Kas* were found to be greater than unity indicating the inhibitory effect of these sulfonamides on the urinary excretion of (−)-mandelic acid.

In order to determine the order of elimination of sulfadiazine, the kinetics of its urinary excretion was studied and from the observed pseudo-first-order process, the overall elimination rate constants were calculated.

The inhibitory effect of (−)-mandelic acid on the urinary excretion of sulfadiazine was also observed.

Since (−)-mandelic acid and sulfadiazine exhibited a mutual inhibitory effect on the kinetics of their urinary excretion, it is concluded that (−)-mandelic acid and the sulfonamides employed in this study probably share the same renal tubular transport system(s) for their excretion in humans.

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ACKNOWLEDGMENT AND ADDRESSES

Received February 26, 1969 from the *College of Pharmacy, Wayne State University, Detroit, MI 48202*

Accepted for publication April 9, 1969.

Abstracted in part from a thesis submitted by F. M. Kamienny to the College of Pharmacy, Wayne State University, in partial fulfillment of Master of Science degree requirements.

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