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Pharmacokinetics of Procainamide in Rats with Extrahepatic Biliary Obstruction

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Abstract □ The pharmacokinetics of the widely used antiarrhythmic agent, procainamide, was studied in rats with extrahepatic biliary obstruction produced by ligation of the common bile duct. Various biological fluids, including plasma, saliva, and urine, were analyzed for procainamide and/or its major metabolite, *N*-acetylprocainamide. Ligation of the common bile duct immediately prior to intravenous administration of 50 mg/kg procainamide did not alter plasma, saliva, or urine concentrations of procainamide, indicating that biliary excretion was of minor importance in the elimination of procainamide. However, bile duct ligation allowed to persist for 4 days significantly elevated plasma, saliva, and urine levels of procainamide. While the increase in urinary procainamide paralleled the increase observed in plasma, salivary concentrations did not. Bile duct ligation did not appear to impair nonmicrosomal acetylation of procainamide, although a significantly greater amount of unchanged drug was found in the urine after 24 hr. Pharmacokinetic analysis *via* the two-compartment open model showed that bile duct ligation caused a decrease in overall clearance from ~61.94 to 28.71 ml/kg/min. This reduction probably resulted from the decreased microsomal metabolism of procainamide. The significant reduction in the apparent volume of distribution from 3.76 to 2.72 liter/kg could be the result of reduced binding sites. There was also a significant increase in the elimination half-life of procainamide from 47.39 to 78.64 min in bile duct ligated rats.

Keyphrases □ Pharmacokinetics—procainamide, rats with extrahepatic biliary obstruction □ Procainamide—pharmacokinetics in rats with extrahepatic biliary obstruction □ Biliary obstruction—extrahepatic, pharmacokinetics of procainamide in rats

Biliary stasis in humans can be caused by tumors, drugs, or various pathophysiological alterations. Many patients with biliary stasis are also critically ill with other serious conditions and therefore are under treatment with a number of pharmacological agents. Inhibition of bile flow and/or biliary excretion may have profound consequences on the pharmacokinetics of drugs, not only because bile can be a route of irreversible elimination, but also because cholestasis may affect microsomal drug metabolism. Tavolini and Guarino (1) demonstrated altered disposition of doxorubicin[1], an antineoplastic agent eliminated extensively in the bile. Other investigators (2–5) demonstrated that microsomal metabolism of a number of drugs that are not extensively excreted in bile is significantly decreased, thus prolonging the elimination half-life ($t_{1/2\beta}$). However, the effects of biliary stasis on the pharmacokinetics of drugs with minimal biliary excretion and minimal microsomal metabolism are unclear. The antiarrhythmic agent procainamide (I) is such a drug. It was the purpose of this investigation to study the effects of cholestasis, induced by mechanical obstruction of the common bile duct, on the pharmacokinetics of I. Salivary levels were measured as an index of drug distribution to the peripheral tissue compartment, and urine levels were assayed to obtain an estimate of the renal clearance of procainamide and the relative urinary metabolic profile.

EXPERIMENTAL

Urine, saliva, and plasma were obtained from a group of normal Wistar rats (weight 190–260 g) for the quantitation of I. Rats were anesthetized with 50 mg/kg ip pentobarbital[2]. Initially, a tracheotomy was performed to facilitate breathing, and then the animals were prepared for collection of parotid saliva according to a previously described method (6). Stimulation of salivation was accomplished by infusing pilocarpine[3] (0.25 mg/ml) into the right brachial artery at a rate of 0.11 ml/min for 90 min. For the administration of I[4] (50 mg/kg), the femoral vein was cannulated. Urine was collected *via* cannulation of the ureters. Urine and saliva were collected over three 30-min collection periods, and 1.5 ml of whole blood was collected at 30, 60, and 90 min postinjection from a cannula placed in the femoral artery. From a separate group of normal rats, plasma was obtained at 30, 60, and 90 min following administration of 50 or 100 mg/kg of I for the quantitation of the acetylated metabolite, *N*-acetylprocainamide (II).

In anticipation of the fact that saliva was to be collected simultaneously with the other biological fluids, preliminary studies were performed to determine the effects of pilocarpine infusion of plasma procainamide concentration and urinary procainamide excretion. Ten control (no pilocarpine) and 10 test rats were used for plasma collection, and another group of five controls and five test animals were used for urine collection.

Extrahepatic biliary obstruction was produced in another group of

[1] Adriamycin.

[2] Nembutal, Abbott Laboratories, North Chicago, Ill.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Squibb Institute for Medical Research, Princeton, N.J.

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Table I—Mean 24-hr Urinary Excretion of Procainamide and *N*-Acetylprocainamide before and after 4-Day Bile Duct Ligation Following Procainamide Administration

Administered dose, mg	Procainamide excreted, %[a]	*N*-Acetylprocainamide excreted, %
	Before Bile Duct Ligation	
11.59 ± 0.90	36.6 ± 3.38	26.2 ± 1.66
	After Bile Duct Ligation	
11.92 ± 0.63	45.5 ± 3.22[b]	30.58 ± 1.12[c]

[a] Percent excretion corrected for 90% recovery. [b] $p < 0.001$. [c] Not significant.

animals by double ligation of the common bile duct following anesthesia with 100 mg/kg of 98% ketamine[5]–2% acepromazine[6] ip. The animals were then sutured and allowed to recover for 4 days. On the fourth day, bile duct ligated animals were prepared for the simultaneous collection of saliva, urine, and plasma after administration of 50 mg/kg iv of I as described above, and the samples were quantitated for I. Plasma samples were also quantitated for II. Animals that did not exhibit jaundice, as evidenced by yellowish skin and bright yellow urine, were not used. Sham operated animals (with laparotomies) were also prepared in a manner identical to actual bile duct ligated animals. A group of six animals were utilized for I determinations immediately after bile duct ligation (Day 0).

To determine the effects of bile duct ligation on the metabolic disposition of I, control rats were injected with 50 mg/kg ip and placed in metabolic cages[7] for 24-hr urine collection. During this period the animals were allowed no food but were given water *ad libitum*. After the 24-hr period of urine collection, a period of 3 days was allowed to elapse to permit the animals to regain a normal metabolic profile. The animals were then anesthetized and the bile ducts were ligated. After 4 days these animals were again administered 50 mg/kg ip of I and placed in metabolic cages for 24-hr urine collection.

Six control and six bile duct ligated animals were prepared for pharmacokinetic studies by performing tracheotomies and cannulating the femoral vein and artery. After administration of 50 mg/kg iv of I, 0.4–0.5 ml of blood was sampled at 5, 15, 30, 45, 60, 90, and 120 min for analysis of the plasma I kinetics. To prevent major perturbation of drug levels, a separate group of six control and six bile duct ligated animals were used to obtain 1- and 5-min levels. The 1-min levels were then included with the values for the other animals for mathematical analysis on the basis of how closely matched the 5-min values were. The two-compartment open pharmacokinetic model was used to describe the kinetics of I, utilizing the biexponential function:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 1)}$$

where C_t is the plasma concentration at time t, A and B are the zero-time intercepts of the fast and slow linear components, respectively, and α and β are the distribution and elimination rate constants, respectively. The pharmacokinetic parameters, A, B, α, and β, were calculated by linear regression of the two linear components of the plasma disappearance curve. Distribution half-life ($t_{1/2\alpha}$), elimination half-life ($t_{1/2\beta}$), volume of distribution (V_d), and clearance (Cl) were calculated by conventional techniques (7).

Analysis of I in all fluid samples and II in urine involved organic extraction of samples into 10 ml of methylene chloride and quantitation *via* a gas chromatograph[8] using dipropylprocainamide[4] as an internal standard as previously described (8). For reasons of sensitivity, plasma of II was quantitated *via* an enzyme-immunoassay method[9]. All statistical determinations of significance were done using the Student's t test. In all cases, except the 24-hr urinary disposition studies (Table I), the unpaired t test was used; in the case of the latter, the paired t test was employed.

RESULTS

Preliminary studies on the effect of pilocarpine infusion indicated that infusion of 0.25 mg/ml pilocarpine at a rate of 0.11 ml/min for 90 min had no effect on the plasma I concentration at any time period studied; however, urinary excretion did show significant decreases during the second and third 30-min collection periods (Table II).

[5] Bristol Inc., Syracuse, N.Y.
[6] Ayerst Laboratories, New York, N.Y.
[7] Wahman Co.
[8] Model 3920, Perkin-Elmer Corp., Norwalk, Conn.
[9] EMIT, Syva Co., Palo Alto, Calif.

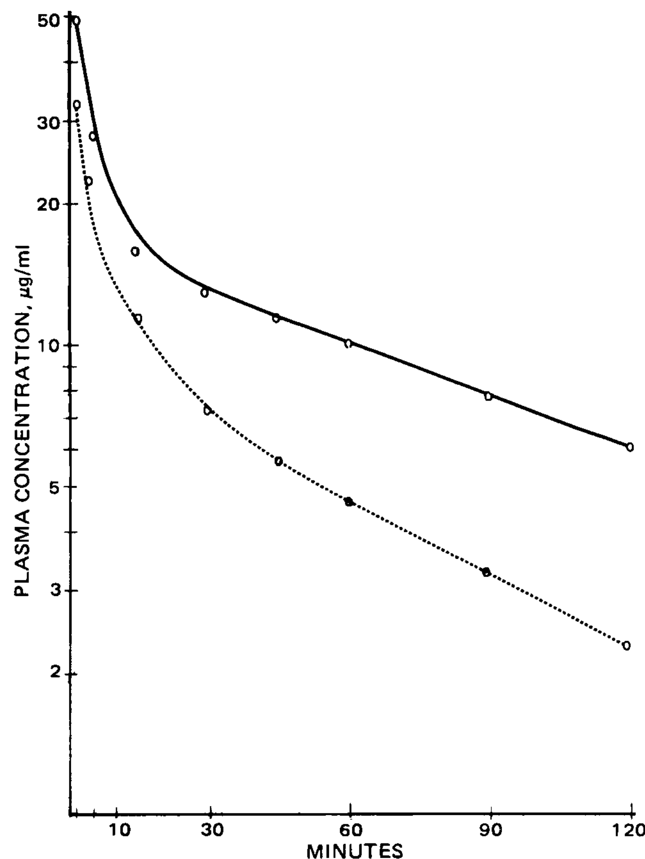

Figure 1—*Plasma disappearance of procainamide.*

The results of GC analysis of plasma, saliva, and urine samples from normal animals receiving a dose of 50 mg/kg iv of I are shown in Table III. The mean I levels in the three biological fluids of sham-operated rats were not significantly different from those of the normal rats at any time period studied. Similarly, in rats whose bile ducts were ligated immediately prior to administration of I (day 0), there were no significant differences in I levels of any of the biological fluids at any time period compared to normal rats. However, when bile duct ligation was allowed to persist for 4 days, significantly higher plasma, saliva, and urine levels were observed at all time periods. Plasma I concentrations increased 100, 125, and 105% at 30, 60, and 90 min, respectively. The increase in urinary excretion paralleled the increase in plasma levels with increases of 94, 125, and 133% over the three respective 30-min collections, indicating that renal excretion is not impaired by bile duct ligation. Salivary I concentrations also increased, but the increases did not parallel those observed in plasma except during the last collection periods. The salivary levels increased ~47, 77, and 125% over the three respective time periods (Table III).

Figure 1 illustrates the disappearance of I from plasma with time for normal animals and animals with 4-day bile duct ligation. The mean pharmacokinetic parameters for both groups are shown in Table IV. Distribution half-life ($t_{1/2\alpha}$) in 4-day bile duct ligated rats is not significantly different compared to normal rats. Elimination half-life ($t_{1/2\beta}$), however, is significantly prolonged ($p < 0.025$). Volume of distribution (V_d) is significantly decreased in 4-day bile duct ligated rats compared to normals ($p < 0.05$). Clearance (Cl) is also decreased by 115% ($p < 0.025$).

To determine whether the observed pharmacokinetic alterations were the result of bile duct ligation-induced metabolic changes, the urinary disposition of I was examined. Table I shows that in the 4-day bile duct ligated rats, the percentage of the drug excreted as II did not change significantly compared to controls. However, the amount of drug excreted unchanged increased significantly ($p < 0.025$). The absence of a change in the production of II during 4-day bile duct ligation was confirmed by measurement of plasma II concentrations in normal and 4-day bile duct ligated rats. In normal rats mean plasma II concentrations at 30, 60, and 90 min were 4.48 ± 0.48, 5.43 ± 0.59, and 7.72 ± 4.77 µg/ml, respectively. In 4-day bile duct ligated rats, mean plasma II concentrations at the same time periods were 4.54 ± 0.36, 7.06 ± 0.72, and 8.96 ± 0.06 µg/ml, respectively.

Table II—Effects of Pilocarpine Infusion on Plasma Procainamide Concentration and Urinary Excretion

	n	30 min		60 min		90 min	
		C[a]	P[b]	C	P	C	P
Plasma procainamide[c]	10	7.49 ± 0.60	8.65 ± 0.73	4.44 ± 0.43	4.53 ± 0.68	3.24 ± 0.28	2.95 ± 0.46
Urinary procainamide[d]	5	0.76 ± 0.17	0.65 ± 0.26	0.50 ± 0.07	0.30 ± 0.10	0.33 ± 0.06	0.20 ± 0.04

[a] C = control (no pilocarpine). [b] P = pilocarpine (0.25 mg/ml) infused at a rate of 0.11 ml/min; the dose was 50 mg/kg iv procainamide. [c] Data expressed as micrograms per milliliter (± *SE*). [d] Data expressed as milligrams (± *SE*).

Table III—Mean Procainamide Concentrations in Various Biological Fluids [a]

Condition	n	Plasma[b] 30 min	60 min	90 min	Saliva[b] 30 min	60 min	90 min	Urine[c] 30 min	60 min	90 min
Normal rats	8–10	8.65 ± 0.73	4.53 ± 0.68	2.95 ± 0.46	16.73 ± 1.69	7.75 ± 1.32	2.82 ± 0.35	0.65 ± 0.26	0.30 ± 0.10	0.20 ± 0.04
Sham-operated rats	5	8.58 ± 0.59	5.42 ± 0.69	3.20 ± 0.33	16.62 ± 1.17	8.00 ± 1.15	3.5 ± 0.41	0.61 ± 0.11	0.33 ± 0.08	0.22 ± 0.06
Day 0 bile duct ligation	6	9.40 ± 0.69	5.43 ± 0.96	4.23 ± 0.85	17.48 ± 1.84	7.56 ± 1.46	3.55 ± 0.60	0.51 ± 0.08	0.31 ± 0.03	0.21 ± 0.05
4-Day bile duct ligation	5–8	17.32 ± 2.15	10.20 ± 2.13	6.06 ± 1.06	24.55 ± 2.23	13.71 ± 1.98	5.68 ± 0.92	1.26 ± 0.19	0.70 ± 0.11	0.46 ± 0.10

[a] Dose of procainamide = 50 mg/kg iv; 0.25 mg/ml pilocarpine infused at a rate of 0.11 ml/min. [b] Data expressed as micrograms per milliliter (± *SE*). [c] Data expressed as milligrams (± *SE*).

It was thought that the higher plasma I levels observed in bile duct ligated animals should result in higher plasma II levels, since more I was apparently available to the liver for acetylation. Since no increase was noted, it was theorized that either bile duct ligation was inhibiting acetylation due to liver damage or that the enzyme responsible for conversion of I to II, N-acetyltransferase, was being saturated. To test these alternative hypotheses, a 100-mg/kg dose of I was administered to control animals to achieve blood levels comparable to those in bile duct ligated animals receiving 50 mg/kg. Plasma I concentrations in normal rats receiving 100 mg/kg iv at 30, 60, and 90 min were 18.22 ± 2.45, 10.93 ± 1.69, and 6.48 ± 1.25 μg/ml, respectively, while plasma II concentrations in those animals were 5.28 ± 0.47, 6.90 ± 0.66, and 7.53 ± 0.75 μg/ml at the three respective time periods. These data show that while plasma I levels in these animals did approximate those seen in bile duct ligated animals receiving half the dose, plasma II levels did not differ from either control or bile duct ligated animals receiving half the dose, suggesting that a saturation of the acetylation process was occurring even at the 50-mg/kg dose.

DISCUSSION

It has been demonstrated (9) that the transport of I into bile is an active process, since the biliary excretion exhibited a concentrative mechanism that was saturable and could be competitively inhibited by other tertiary and quaternary amines. However, since ligation of the bile duct immediately prior to intravenous administration of I did not significantly elevate plasma, saliva, or urine levels of the drug at any time period studied, it is apparent that biliary excretion of I is a minor component in its overall elimination (Table III). This is in agreement with previous findings (10), which state that renal excretion is the major route of elimination for this drug. Furthermore, simply shutting off the biliary route of excretion cannot be invoked as the primary explanation for pharmacokinetic alterations observed in 4-day bile duct ligation in the case of I. However, the small amount of I that normally appears in bile, which according to a previous report (11) amounts to 3–5% of the administrated dose, would be reabsorbed and contribute to elevated plasma levels.

The most logical hypothesis concerning the underlying mechanism for the altered kinetics would be that the metabolism of I was being inhibited due to liver damage resulting from the reflux of bile acids in a manner similar to that proposed for a number of drugs by various investigators (2–5) (*i.e.*, destruction or decreased synthesis of cytochrome P_{450}).

Table IV—Mean Pharmacokinetic Parameters of Procainamide in Normal and 4-Day Bile Duct Ligated Rats [a]

Parameters	Control	Bile Duct Ligated
$t_{1/2\alpha}$, min ± SE	4.87 ± 1.06	2.95 ± 0.90
$t_{1/2\beta}$, min ± SE[b]	47.39 ± 3.81	78.64 ± 13.13
V_d, liter/kg ± SE[c]	3.72 ± 0.47	2.76 ± 0.21
Cl, ml/kg/min ± SE[b]	61.94 ± 13.68	28.71 ± 3.80

[a] Dose of procainamide was 50 mg/kg iv; n = 6 rats. [b] $p < 0.025$. [c] $p < 0.05$.

However, the 24-hr urinary II levels as well as plasma II levels indicate that there is no difference in the amount of II produced by 4-day bile duct ligated rats compared to normal rats. Since II, the major metabolite of I, is a product of a nonmicrosomal metabolism (*i.e.*, not dependent on the mixed-function oxidase system), it appears that nonmicrosomal acetylation of I is relatively resistant to inhibition by ligation of the common bile duct. Furthermore, the studies with 100 mg/kg I in normal rats indicate that the acetylation enzyme, N-acetyltransferase, is saturable at the 50-mg/kg dose level of I, and thus, the activity of the enzyme in normal and 4-day bile duct ligated animals is apparently the same. These results also indicate that decreased production of II is not the primary mechanism responsible for the elevation of I concentrations in the various biological fluids.

However, inhibition of microsomal metabolism may play a contributing role in the altered kinetics of I, since a small percentage of the drug undergoes some microsomal metabolism. Recently, two other metabolites of I were found (12): desethylprocainamide and N-acetyldesethylprocainamide; the former being the primary intermediate in the formation of the latter. Since bile duct ligation has been shown to inhibit microsomal metabolism and the data in this study show significantly greater amounts of unchanged drug excreted (Table I), it is probable that these metabolites are not produced to the normal extent, thereby contributing to higher plasma levels of the parent compound. This may explain the prolonged $t_{1/2\beta}$ calculated in this study.

Since elimination by the biliary route of excretion or decreased acetylation apparently are not the major mechanisms underlying the bile duct ligation-induced alteration in the pharmacokinetics of I, other mechanisms must be operative. Calculation of various pharmacokinetic parameters (Table IV) revealed that there is a significant reduction in V_d of I in 4-day bile duct ligated rats. The reduction in V_d suggests that the drug is not leaving the central compartment as readily as in normal rats, thus contributing to higher than normal plasma concentrations. The fact that there was no significant difference in the calculated $t_{1/2\alpha}$ indicates that the rate of distribution is not affected by 4-day bile duct ligation, only the extent of the distribution is. The idea that bile duct ligation can induce a change in drug distribution has also been advanced in a previous report (2) where it was postulated that increased thiopental sleeping times may be due to this phenomenon. The decreased distribution to the peripheral compartment observed in this study is borne out by the salivary excretion data which showed that while there was an increase in saliva I concentration, it was not of the same magnitude as the increase in plasma except at the 90-min mark. One possible explanation for the decreased V_d may involve elevated bilirubin levels, which result from bile duct ligation (13). High levels of bilirubin have been associated with displacement of drugs from plasma proteins. Since plasma protein binding of I is negligible (10–15%) but tissue protein binding is extensive, it is possible that hyperbilirubinemia caused a significant reduction in tissue binding. Such an idea has been suggested previously (14). A significant decrease in tissue binding of a drug, which is normally bound to tissues extensively, would cause a decrease in the V_d of the drug.

Thus, although biliary excretion of I is of minor importance as a route of elimination, this study demonstrates that extrahepatic biliary obstruction induced by bile duct ligation can still lead to a significant al-

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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