Relationship between *In Vitro* Dissolution Rates, Solubilities, and LT_{50}'s in Mice of Some Salts of Benzphetamine and Etryptamine

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Salt formation of benzphetamine and etryptamine as a potential means of obtaining sustained release and/or prolonged action was studied by measuring the median lethal time (LT_{50}) in mice and its relationship to *in vitro* dissolution rates and solubilities. Both the LT_{50} and LD_{50} increase as the *in vitro* dissolution rate, determined at pH 7.2, decreases. An empirical relationship was found between the LT_{50} and the *in vitro* dissolution rate at pH 7.2. The experimental data suggest that salt formation is a potentially useful means of approaching development of sustained- or prolonged-action dosage forms of certain drugs.

THE PURPOSES of this study were: (*a*) to investigate salt formation as a means of obtaining sustained release and/or prolonged action, and (*b*) to investigate relationships between toxicity data and *in vitro* dissolution rates and solubilities. The relative toxicities of a series of salts of a drug reflect the rate of absorption providing the salt-forming agents are relatively nontoxic. When absorption is rate-limited by dissolution of the salt in the gastrointestinal tract, as will be the case with slowly soluble salts, the toxicity of a slowly dissolving salt will most probably be lower than that of a more rapidly dissolving salt. A similar concept was applied by Nelson (1) in which the clinical effects noticed with various theophylline salts were explained on the basis of dissolution rates. Becker and Swift (2) showed that sustained-release properties of drug resinates could be evaluated by observing the median lethal time of death (LT_{50}) of the drug resinate. Greater sustained or slow release of drug provided an increased LT_{50} and an increased LD_{50} resulting from slower absorption. *In vitro* release of drug from other resinates (2, 3) and from sustained-release capsules[1] (4) was found to correlate with toxicity and blood levels in animals. Shenoy, Grice, and Campbell (5) found the determination of the LD_{50} of a sustained-release formulation to be a very useful method of assessing sustained-release preparations; however, the limitation of the method was the inability to distinguish between sustained and/or slow release and nonavailability of drug. They found that supposed sustained-release products which failed to show sustained release by means of urinary excretion studies in human subjects also showed no sustained release in LT_{50} studies in animals.

The compounds selected for this study were etryptamine[2] and benzphetamine,[3] both of which are basic amino compounds with apparent pKa values in water of 8.8 and 9.3, respectively. Both amine bases have low water solubilities but form highly soluble hydrochlorides.

EXPERIMENTAL

Preparation of Salts.—Salts of the amine drugs were prepared by (*a*) the reaction of the amine hydrochloride with an alkali salt of an acid in aqueous or aqueous-organic solvent mixtures, or by (*b*) reaction of the amine base with a free acid in an appropriate solvent. Elemental analysis and equivalent weight confirmed the stoichiometry of the salt.

Determination of LT_{50} and LD_{50}.—Each salt was ground to a fine powder in a mortar, suspended in 1.0% carboxymethylcellulose by means of a tissue grinder, and intubated orally into ten starved mice. The amount of salt administered was equivalent to that amount which just produced 100% death in an acute toxicity study. In the benzphetamine series it was that quantity equivalent to 400 mg./Kg. benzphetamine hydrochloride, whereas in the etryptamine series it was that quantity equivalent to 124 mg./Kg. etryptamine acetate. The death time of each animal was recorded to the nearest minute.

The LT_{50} was estimated as the time midway between the times to death of the fifth and sixth animals. This method is an alternative to that in which the intersection of the 50 percentile point with a plot of cumulative percentage mortality on log-probability graph paper is taken as the LT_{50} (2–5). This method was chosen since many sets of such mortality data are not truly log-normally distributed.

Determination of *in vitro* Dissolution Rates and Solubilities.—The solubilities were determined by: (*a*) equilibration of excess compound with water for

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[1] Spansule, trade-marked name of Smith Kline and French's sustained-release capsule.

[2] Etryptamine (Monase, as the acetate) is 3-(2-aminobutyl)indole.

[3] Benzphetamine (Didrex, as the hydrochloride) is (+)N-benzyl-N, α-dimethylphenethylamine.

24 hours at room temperature and determination of the amount in solution, or by (*b*) visual observation of the amount which just dissolves upon equilibration as above.

The dissolution rates were determined by the hanging pellet method of Nelson (6). The pellets were 13 mm. in diameter and were compressed at 20,000 p.s.i. The dissolution medium was 500 ml. of 0.1 *N* hydrochloric acid or 0.2 *M* tris(hydroxymethyl) aminomethane solution adjusted to pH 7.2 with hydrochloric acid and maintained at 37°.

RESULTS AND DISCUSSION

Tables I and II show the various salts of benzphetamine and etryptamine, respectively, arranged in order of increasing LT_{50}. The cumulative death data used in determining the LT_{50} are shown in Tables III and IV. Inspection of Tables I and II shows that the *in vitro* dissolution rates determined at pH 7.2 correlate much better with LT_{50} than do either the water solubilities or dissolution rates determined at pH 1. There is poor correlation of water solubility with dissolution rate, probably because the water solubility is an equilibrium condition at an uncontrolled pH whereas the dissolution rate is a dynamic property dependent upon many factors (7). The polygalacturonate salt of benzphetamine is an example of a salt with a very high water solubility but low dissolution rates. An accurate determination of the solubility of this salt was hindered by the high viscosity of the resultant solution. The seeming anomalously low dissolution rates of this salt are probably due to the high viscosity of the diffusion layer.

Some salts, notably the 8-nitrotheophyllinates, diliturates, bis-methylene-salicylates, and pamoates showed unusual pellet dissolution at pH 1.0. It was found that a layer of the insoluble acid, resulting from hydrolysis of the salt, was formed on the surface of the pellet while the protonated amine diffused through this barrier.

Only a few of the LD_{50}'s were determined (Table I). However, for those available there appears to be a qualitative relationship between LD_{50}, LT_{50}, and dissolution rates at pH 7.2; both the LT_{50} and LD_{50} increase as the dissolution rate decreases. It appears then that one should always use the most rapidly dissolving salt or other form of a drug in determining the intrinsic acute LD_{50} of the drug; conversely, the apparent acute toxicity may be decreased by employing a slowly dissolving form of the drug.

When the LT_{50} of each salt was plotted against the *in vitro* dissolution rate, determined at pH 7.2,

TABLE I.—BENZPHETAMINE SALTS

Salt	M.p., °C.	Equivalent Water Solubility,[a] mg./ml.	Equivalent Rate of Dissolution,[b] mg./cm.²/hr. pH 1.0	pH 7.2	LT_{50}[c]	90% Confidence Limits of LT_{50}[d]	Equivalent LD_{50},[e] mg./Kg.
Hydrochloride	131–133	434–781	564	542	10	8–12	174
					8	6–10	...
Diliturate	133–134	5.01	...	5.5	12	11–13	...
Polygalacturonate	...	...	3.7	9.5	14.5	13–18	193
8-Nitrotheophyllinate	173–174	0.66	3.26	0.86	16.5	14–22	225
Barbiturate	203–205	4.36	94.5	4.30	27	21–322	248
Methylene-bis-salicylate[f]	89–91	0.15	0.013	0.20	30.5	13–139	...
2,4-Dihydroxybenzoate	73–75	12.9	51	1.6	98	30–1117	...
Pamoate[f]	101–103	0.06	3.6	0.07	804	169–1375+	278

[a] Equivalent water solubility = solubility of the salt × $\frac{\text{mol. wt. of the amine base}}{\text{mol. wt. of the salt}}$. [b] Equivalent dissolution rate = apparent dissolution rate × $\frac{\text{mol. wt. of the amine base}}{\text{mol. wt. of the salt}}$. [c] Median lethal time estimated as the time midway between the times to death of the fifth and sixth (of 10) mice. [d] Estimated from the time to death of the third and eighth (of ten) mice to die. [e] Equivalent LD_{50} = LD_{50} of the salt × $\frac{\text{mol. wt. of the amine base}}{\text{mol. wt. of the salt}}$. [f] The stoichiometry of these salts is 2 amine:1 acid.

TABLE II.—ETRYPTAMINE SALTS

Salt	M.p., °C.	Equivalent Water Solubility,[a] mg./ml.	Equivalent Rate of Dissolution,[b] mg./cm.²/hr. pH 1.0	pH 7.2	LT_{50}[c]	90% Confidence Limits of LT_{50}[d]
Acetate	165–166	88.8	336	264	3	2–3
Free base	104–105	0.51	97.8	24.5	4	4–5
8-Chlorotheophyllinate	212–214	3–5	157	40.9	4.5	4–5
Barbiturate	257–258	5–10	112	11.4	12.5	10–14
Pamoate[b]	136–144	0.03–0.12	7.9	0.098	111	37–124
8-Nitrotheophyllinate	214–217	0.25	5.56	0.49	209	119–277

[a, b, c, and d] See footnotes in Table I.

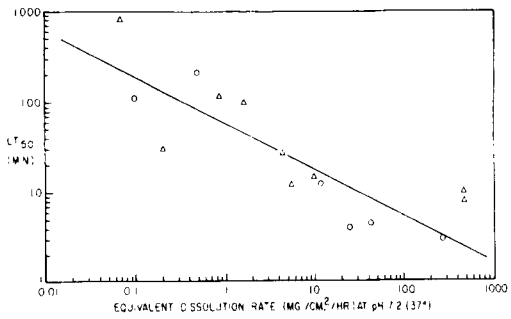

Fig. 1.—A plot of LT_{50} in mice against equivalent dissolution rate at pH 7.2 for etryptamine and benzphetamine salts. Δ, Benzphetamine salts; O, etryptamine salts.

on log-log graph paper, the points appeared to be linearly distributed. Estimation of the least squares regression line indicated the slope of the line was approximately −0.5. Figure 1 is such a plot with the solid line having a slope of −0.5. It follows that for the salts studied, the LT_{50} is inversely related to the square root of the equivalent *in vitro* dissolution rate determined at pH 7.2. This treatment is similar to that of Sturtevant, *et al.* (4), in which linear relationships were found between the logarithm of ΔLT_{50} and the logarithm of the *in vitro* release rates of various sustained-release amphetamine bead preparations.

SUMMARY AND CONCLUSIONS

1. Series of salts of benzphetamine and etryptamine were prepared.

2. The water solubility, *in vitro* dissolution rates determined in aqueous media having a pH of 1.0 and 7.2, and the median lethal time of death (LT_{50}) were determined for each salt. The LD_{50} (mice) of five of the benzphetamine salts, following oral administration, were also determined.

3. The experimental data show that the times to death of mice following a lethal dose and the LD_{50}'s of the various salts are related to the rates of dissolution of the salts as determined by a standardized *in vitro* test.

4. Regression analysis indicated that the LT_{50} is inversely related to the square root of the equivalent *in vitro* rate of dissolution determined at pH 7.2 for the salts studied.

5. The water solubilities and the *in vitro* rates of dissolution determined at pH 1.0 did not correlate nearly as well with the toxicity data as did the *in vitro* rates of dissolution determined at pH 7.2.

TABLE III.—CUMULATIVE DEATH DATA OF BENZPHETAMINE SALTS

	Times, to Death of Mice for Benzphetamine, min.							
% Mortality	Hydrochloride	Dilitrate	Polygalacturonate	8-Nitrotheophyllinate	Barbiturate	Methylene bis-Salicylate	2,4-Dihydroxybenzoate	Pamoate
100	11	35	60	209	...	195	7200	...
90	...	21	20	...	...	194	...	...
80	10	13	18	22	322	139	1117	...
70	...	12	17	21	28	41	162	1375
60	8	...	15	18	27	39	161	...
50	...	...	14	15	...	22	35	234
40	7	11	13	14	25	21	33	206
30	6	...	...	...	21	13	30	169
20	5	...	11	...	...	...	13	162
10	3	10	9	...	19	...	10	121

TABLE IV.—CUMULATIVE DEATH DATA FOR ETRYPTAMINE SALTS

	Times to Death of Mice for Etryptamine, min.					
% Mortality	Acetate	Base	8-Chlorotheophyllinate	Barbiturate	Pamoate	8-Nitrotheophyllinate
100	4	6	5	15	276	308
90	3	5	...	14	153	280
80	...	4	...	...	124	277
70	...	...	...	13	114	249
60	...	...	...	...	112	233
50	...	...	4	12	110	186
40	2	...	...	10	47	175
30	...	...	...	...	37	119
20	...	...	...	...	34	...
10	...	3	...	9	...	115

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Excretion Patterns of Phenothiazine-S^{35} Compounds in Rats

Effect of Change in Structure on Metabolism

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The excretion patterns of five S^{35}-labeled phenothiazines were compared in rats following the oral administration of pharmacologically active doses. Substitution in the 2 position in the phenothiazine ring did not have any apparent effect upon the S^{35} excretion pattern. Differences in the structure of the side chain did have an effect upon the mode of S^{35} excretion. The urinary S^{35} excretion decreased and the fecal S^{35} excretion correspondingly increased as the side-chain structure was changed from 3-dimethylaminopropyl to 3-dimethylamino-2-methylpropyl to 4-methyl-1-piperazinylpropyl. At a dosage level of 9.0 mg. of free base/Kg. in rats, the urinary S^{35} excretion following the oral administration of compound II [2-chloro-10-(3-dimethylaminopropyl)phenothiazine-S^{35} hydrochloride] was twice as great as the urinary S^{35} excretion following the oral administration of compound III [10-[3-(4-methyl-1-piperazinyl)-propyl]-2-trifluoromethylphenothiazine-S^{35} dihydrochloride]. The former compound contained a chlorine atom in the 2 position of the phenothiazine nucleus, while the latter contained a trifluoromethyl grouping at this position. The increased dosage level (9.0 mg. of free base/Kg.) did not appreciably change the S^{35} excretion pattern of compound IV [10-(3-dimethylamino-2-methylpropyl)phenothiazine-S^{35} tartrate] and compound V [10-(3-dimethylamino-2-methylpropyl)-2-trifluoromethylphenothiazine-S^{35} hydrochloride].

ALTHOUGH there are reports in the literature on the urinary excretion pattern of chlorpromazine [2-chloro-10-(3-dimethylaminopropyl)-phenothiazine hydrochloride] there are few comparative studies of the excretion patterns of various phenothiazines. Christensen and Wase (1), using mice as the experimental animals, were the first to report on the urinary and fecal excretion of chlorpromazine-S^{35}. In a preliminary communication Fyodorov and Shnol (2) reported that, following the oral administration of chlorpromazine-S^{35} (aminazine-S^{35}) to rats, 16–17% of the radioactivity was excreted in the urine while approximately 76% was excreted in the feces. In other communications Fyodorov (3, 4) reported on the fate of three phenothiazine compounds in the organism: aminazine-S^{35}, promazine-S^{35}, and chlormeprazine-S^{35}. In his studies with aminazine-S^{35}, Fyodorov found a high concentration of radioactivity in the bile following oral administration. He reported (5) that a large part of the aminazine did not enter into the general blood stream but circulates in a closed circuit: intestine, portal vein, liver, bile, and back to the intestine being eliminated gradually in the feces.

In our laboratories it was shown that, following the intraduodenal administration of chlorpromazine to anesthetized dogs, the bile contained a high percentage of the drug and its metabolites (6). In similar experiments in dogs with three S^{35}-labeled phenothiazines, it was found that the phenothiazines were excreted in large but varying amounts *via* the biliary route. In light of these results it was decided to study the excretion patterns of five phenothiazine-S^{35} compounds in normal rats to see what effect changes in nucleus substitution and/or in the side chain would have upon urinary and fecal excretion following oral administration. Because of the large biliary excretion of the phenothiazines, it was recognized that the fecal pattern of each drug would not be a measure of unabsorbed drug but rather an indication of the amount of phenothiazine entering into the closed circuit system.

The structures of the phenothiazine-S^{35} compounds utilized are shown in Fig. 1.

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