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Preformulation Investigation I: Relation of Salt Forms and Biological Activity of an Experimental Antihypertensive

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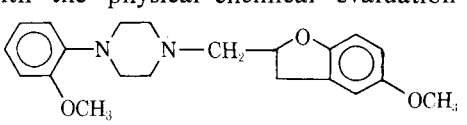
Abstract □ The intrinsic dissolution rates of monohydrochloride, dihydrochloride, and disulfate salts and free base of an antihypertensive were studied. The comparative hypotensive activity of these compounds was studied in anesthetized dogs. The hypotensive responses were also compared to a large number of renal hypertensive dogs who had received a placebo treatment. The results from the anesthetized dog study indicate that the hypotensive potencies of the three salts do not differ from each other; however, the intrinsic dissolution rates of the dihydrochloride and disulfate salts are 54 and 42 times greater, respectively, than that observed with the monohydrochloride salt. These findings seem to indicate the oversensitivity of the *in vitro* dissolution method in reflecting the *in vivo* hypotensive activity of the compound. The free base failed to produce a significant dose–response curve in the anesthetized dog study as well as in the renal hypertensive dog study. This is attributed and correlated to the fact that the *in vitro* dissolution rate of the free base is very low in magnitude as compared to its corresponding salts. The results point out that in searching for a potential candidate of a drug substance, it is advisable to study routinely the effect of the salt form on the biological response.

Keyphrases □ 1-(2,3-Dihydro-5-methoxybenzo[*b*]furan-2-ylmethyl)-4-(*o*-methoxyphenyl)piperazine and salts—dissolution properties, comparative antihypertensive activity in dogs □ Antihypertensive activity—1-(2,3-dihydro-5-methoxybenzo[*b*]furan-2-ylmethyl)-4-(*o*-methoxyphenyl)piperazine and salts, dogs □ Dissolution profiles—free base and salts of an experimental antihypertensive □ Structure–activity relationships—free base and salts of an experimental antihypertensive

The dissolution rates, with respect to pH, of several weak acids and their sodium salts in media representing GI fluids were first investigated by Nelson (1). The salts were found to dissolve much more rapidly than the corresponding free acids. Similar but further elaborated studies on the dissolution kinetics in reactive media were reported by Higuchi and his coworkers (2–4). Salt formation of benzphetamine and etryptamine as a potential means of obtaining timed release and/or prolonged action was studied (5) by measuring the median lethal time in mice and its relationship to *in vitro* dissolution rates and solubilities. It was demonstrated (6) that the dissolution rate of the weak acid, theophylline, was markedly enhanced by employing its amine or alkanolamine salts. The differences in theophylline blood levels after oral administration of the salts in a clinical study were suggested to be related to differences in dissolution rate.

It has been documented that the therapeutic and absorption efficacy of various drugs can be affected by various factors. Consequently, with the emphasis on drug bioavailability in the design of a dosage form, it is pertinent to investigate the various physical-chemical properties of the compound prior to preliminary formulation work. The scope of the preformulation work was aptly outlined by Simons (7). The present study deals with the physical-chemical evaluation of an



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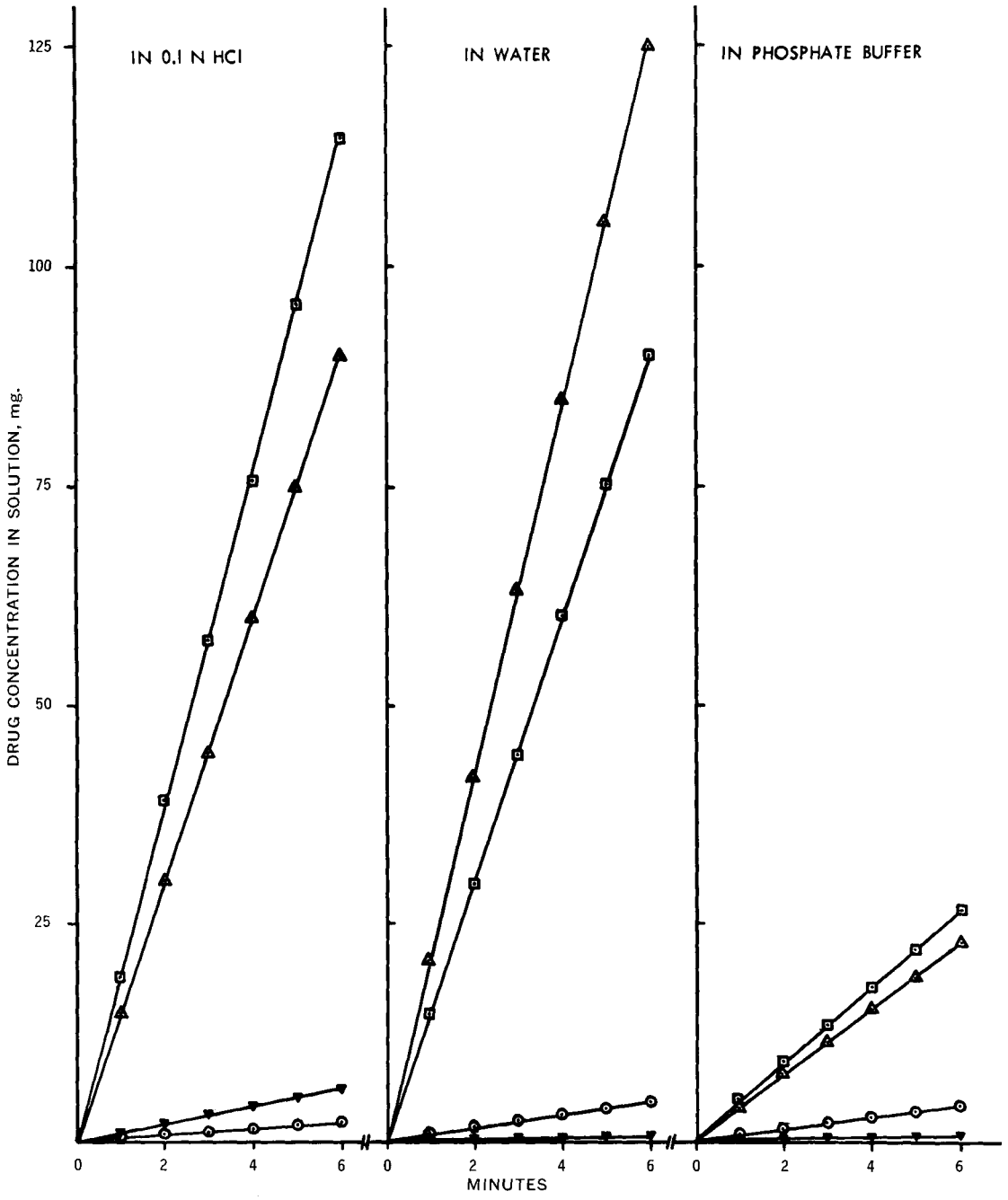

Figure 1—*Plots illustrating the intrinsic dissolution profiles of the dihydrochloride* (△), *monohydrochloride* (○), *and disulfate* (□) *salts and free base* (▽) *of I in 0.1* N *hydrochloric acid, water, and phosphate buffer at pH 7.3.*

experimental antihypertensive compound, 1-(2,3-dihydro-5-methoxybenzo[*b*]furan-2-ylmethyl)-4-(*o*-methoxyphenyl)piperazine (I)[1].

In this paper, emphasis is placed on: (*a*) the physical-chemical characteristics, especially the dissolution properties, of three salt forms of this potential antihypertensive compound as compared to its free base; and (*b*) the relationship between the salt form and biological activity in dogs.

EXPERIMENTAL

Determination of Intrinsic Dissolution Rate—By using the dissolution apparatus of Wood *et al.* (8), the dissolution behavior of the compound under investigation was studied in 300 ml. of 0.1 *N* hydrochloric acid, triple-distilled water, and pH 7.3 phosphate buffer in an 800-ml. round-bottom flask. Three hundred milligrams of the compound was accurately weighed and transferred into a die-punch assembly. The powders were compressed with the Carver press under a pressure of 1500 p.s.i. to form a flat-surfaced disk having a diameter of 0.08 cm. (0.031 in.). The lower surface of the compressed disk was positioned 2 cm. below the surface of the dissolution fluid, which was maintained at 37°. A three-blade stirrer, 4.3 cm. in diameter, was used to stir the dissolution media. The rate of stirring was 40 r.p.m. Aliquots of the dissolution fluid were withdrawn, filtered, and assayed spectrophotometrically at 282 nm. at specific time intervals. The results obtained are depicted in Fig. 1, and the intrinsic dissolution rate of each compound was calculated from the slope of each curve and summarized in Table I.

Comparative Hypotensive Activity—The monohydrochloride, dihydrochloride, and disulfate salts and free base of Compound I were studied in the anesthetized dog for hypotensive activity. The com-

[1] Su-17770.

Table I—Intrinsic Dissolution Rate of I

Compound I Form	Apparent Intrinsic Dissolution Rate, mg. cm.²/min. 0.1 *N* HCl	Water	Phosphate Buffer
Disulfate salt	32.00	30.00	7.33
Dihydrochloride salt	25.00	35.33	6.33
Monohydrochloride salt	0.59	1.13	1.17
Free base	1.67	0.07	0.12

pounds were introduced into the lumen of a loop of the small intestine. The doses employed were molar equivalent doses to 0.56 and 1.67 mg./kg. of the dihydrochloride salt, as shown in Table II. The ratio between graded doses was always a factor of three. There were five to eight dogs in each experimental group, and the results obtained are shown in Table II. By means of statistical analysis, the results obtained with the monohydrochloride and disulfate salts and free base were compared to the results obtained with the dihydrochloride salt.

In a second study, the hypotensive responses produced by the three salts and the free base of I were compared to a large number of renal hypertensive dogs who had received a placebo. Table III shows a summary of blood pressures recorded initially and following 12 days of treatment.

RESULTS AND DISCUSSION

As shown in Fig. 1, the intrinsic dissolution rates of the disulfate and dihydrochloride salts were much greater than for the corresponding monohydrochloride salt and free base when the dissolution medium employed was 0.1 *N* hydrochloric acid, distilled water, or pH 7.3 phosphate buffer. Determinations were reproducible to within 4% of the mean of four runs. No disintegration of the compressed disk was observed during and after the dissolution tests. The difference in the order of magnitude of the intrinsic dissolution rates among these compounds is clearly illustrated in Table I. For example, the intrinsic rate of disulfate salt in 0.1 *N* hydrochloric acid was about 54 and 19 times greater than that of the monohydrochloride salt and free base, respectively; the intrinsic dissolution rate of the dihydrochloride salt in phosphate buffer was approximately 53 and 5.4 times greater than that of the free base and monohydrochloride salt, respectively. The observed differences in the intrinsic dissolution rate between the salts and between any salt and the free base were attributed to the properties of the diffusion layer surrounding a dissolving solid.

It was observed that both the dihydrochloride and the disulfate salts were readily soluble in water to the extent of about 20%. However, the much less soluble monohydrochloride salt was found to precipitate out of the solution containing dihydrochloride salt within 8 hr. at room temperature. Even in the solid state, gaseous hydrochloric acid could be detected with Congo red paper when dihydrochloride salt was stored in the closed container. For the solution containing disulfate salt, the monosulfate salt was found to come out of solution only after 24 hr. at room temperature. The monohydrochloride salt gave off no gaseous hydrochloride and was considerably less soluble in water. The equilibrium solubility of the monohydrochloride salt at 37° was 1.60% in water, 0.73% in 0.1 *N* hydrochloric acid, and 0.98% in phosphate buffer.

The comparative hypotensive activity of the various salt forms in anesthetized dogs is shown in Table II. The results indicate that the potencies of the monohydrochloride and the disulfate salts do not differ from the potency of the dihydrochloride salt within 95% confidence limits. The free base failed to produce a significant dose–response curve. The hypotensive response was slight and was not dose dependent. To confirm this finding, further molar equivalent doses of the dihydrochloride (1.67 mg./kg.), the monohydrochloride (1.53 mg./kg.), and the disulfate (2.15 mg./kg.) salts and the free base (1.38 mg./kg.) were administered to groups of six renal hypertensive dogs for 12 consecutive days. Blood pressure and heart rate were monitored and recorded on the 1st, 4th, 8th, and 12th days of treatment. By means of statistical analysis, it was found that the hypotensive responses produced by a dose of the monohydrochloride and disulfate salts did not differ significantly from that of the dihydrochloride salt. However, the free base did not produce a significant hypotensive response in the renal hypertensive dogs.

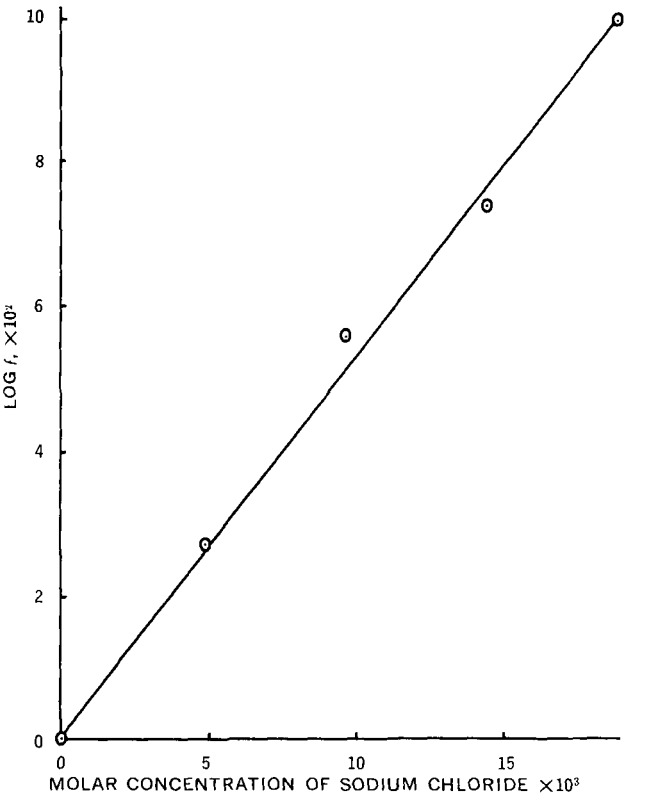

Figure 2—*Effect of salt concentration on the activity coefficient of the monohydrochloride salt of I at 37°.*

The *in vivo* results shown in Table II do not clearly allow one to select the best salt form. Although the difference in hypotensive activities of these three salt forms is not demonstrable by the *in vivo* method, the *in vitro* dissolution rate method (Fig. 1) does afford distinction among the salts of this compound. For example, the intrinsic dissolution rates of the disulfate and dihydrochloride salts are 54 and 42 times greater, respectively, than the rate for the monohydrochloride in 0.1 *N* hydrochloric acid. The findings seem to indicate the oversensitivity of the *in vitro* dissolution method in reflecting the *in vivo* hypotensive activity of the compound.

As shown in Fig. 1 and Table I, the hydrochloride salt of the compound exhibited a greater dissolution rate than the free base in distilled water; the order was reversed when the dissolution medium

Table II—Comparative Oral Hypotensive Activity of I

	Compound								
	Dihydrochloride Salt		Monohydrochloride Salt		Disulfate Salt			Free Base		
Dose, mg./kg.	0.56	1.67	0.51	1.53	0.72	2.15	6.45	0.46	1.38	4.14
Number of dogs	6	6	6	8	5	6	6	6	6	6
Relative potency	1.00		1.59		0.68			Not valid		
95% confidence limits	—		0.81–7.66		0.28–2.51			Common slope not significant		

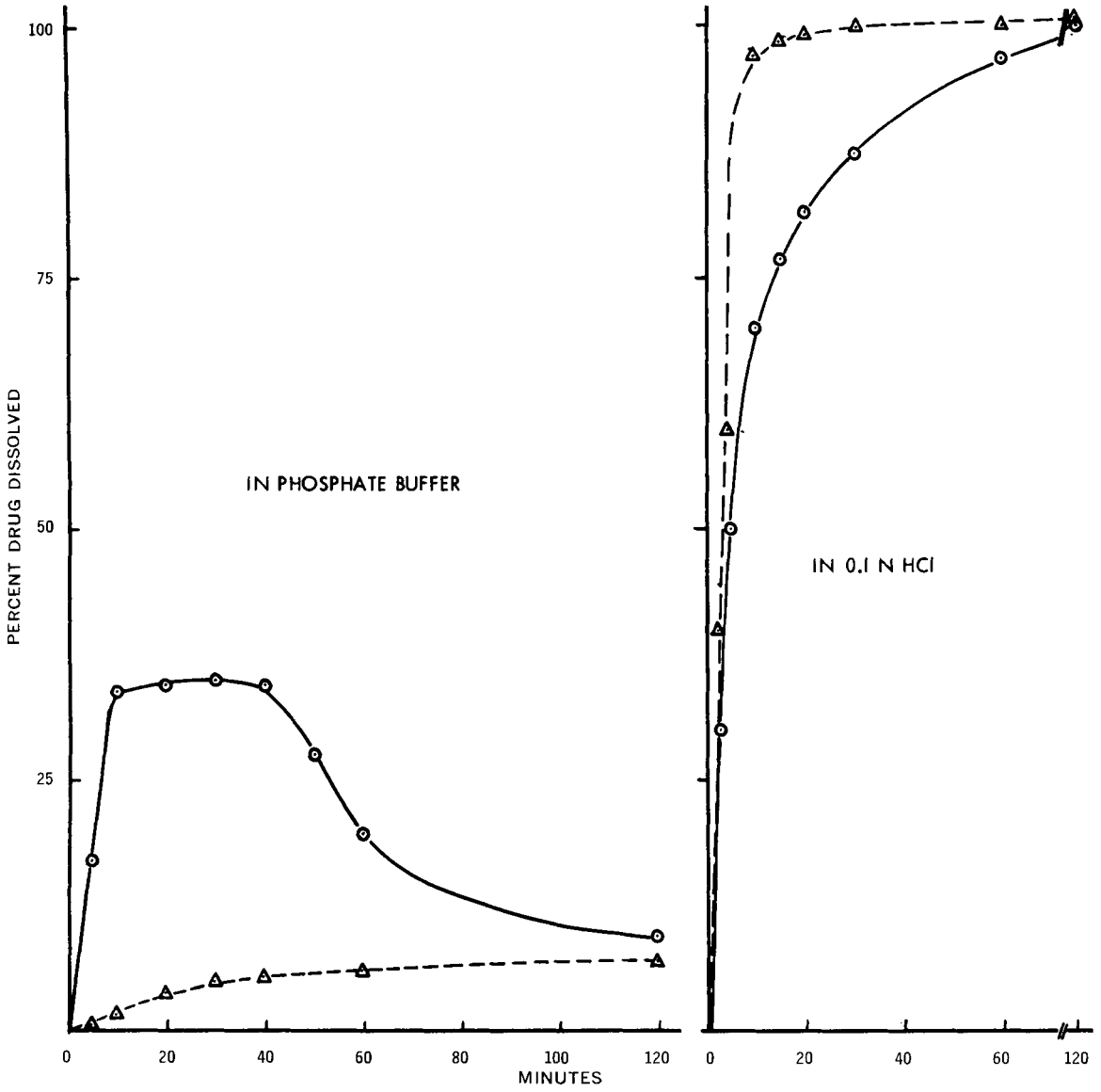

Figure 3—*Dissolution profiles of the encapsulated free base* (- - -) *and the monohydrochloride salt* (——) *of I in 0.1* N *hydrochloric acid and phosphate buffer at pH 7.3.*

was changed from water to 0.1 *N* hydrochloric acid. This is attributed to the common-ion effect, and the experimental evidence is shown in Fig. 2. Aqueous solutions of sodium chloride in 0.1 *N* hydrochloric acid were prepared in concentrations up to 0.02 *M*, and the equilibrium solubility of the monohydrochloride salt in these solutions was studied at 37°. The empirical equation of Setschenow (9) used in expressing the extent of the salt effect is:

$$\log f = \log \frac{S_0}{S} = kC \qquad \text{(Eq. 1)}$$

where S and S_0 are the solubilities of the electrolyte in solvent with and without the presence of salt, respectively; and C is the molar concentration of the salt solution. The ratio S_0/S is equal to f, the activity coefficient of the nonelectrolyte in the salt solution. The overall salting-out constant, k, can be derived from the slope of a plot of $\log f$ *versus* C. As shown in Fig. 2, a straight-line relationship was observed when $\log f$ was plotted *versus* molar concentration of sodium chloride. The salting-out constant is estimated to be 5.3.

Table III indicates the hypotensive responses produced by the three salts in renal hypertensive dogs as compared to a large number of renal hypertensive dogs who received a placebo. The dihydrochloride and disulfate salts produced more marked hypotensive effects than the monohydrochloride salt. The monohydrochloride salt produced a 20-mm. decrease in mean blood pressure, while the dihydrochloride and disulfate salts produced 37- and 29-mm. decreases in mean blood pressure, respectively. The percentage decreases of the mean blood pressure after 12 days of treatment as compared to that found at zero time were 19, 15, and 10% when the dihydrochloride, disulfate, and monohydrochloride salts were employed, respectively. The relative order of decrease in hypoten-

Table III—Hypotensive Response of I in Renal Hypertensive Dogs

Compound	Dose, mg./kg.	Number of Dogs	Mean Blood Pressure, mm. Hg: Zero Time	Mean Blood Pressure, mm. Hg: 12 Days	% Reduction	*p*
Placebo	—	23	178	175	2	0.001
Dihydrochloride salt	1.67	6	193	156	19	
Placebo	—	17	179	175	2	0.01
Monohydrochloride salt	1.53	12	195	175	10	
Placebo	—	23	183	180	2	0.001
Disulfate salt	2.15	6	188	159	15	

sive responses of the dihydrochloride, disulfate, and monohydrochloride salts appears to have a similar rank order relationship with the intrinsic dissolution rates of these compounds, obtained with distilled water as the dissolution medium.

As mentioned previously, the free base failed to produce a significant dose–response curve in the anesthetized dog study. In the unanesthetized renal hypertensive dog study, the free base also failed to produce a significant hypotensive response. This *in vivo* finding is attributed and correlated to the fact that the *in vitro* intrinsic dissolution rates of the free base in water and phosphate buffer (Fig. 1) are found to be very low in magnitude as compared to the salts of this compound. For further illustration of this *in vivo–in vitro* correlation, dissolution profiles of the free base and the monohydrochloride salt in capsule form without the presence of excipients were investigated. Approximately 100 mg. of the compound was accurately weighed and transferred into a Number 3 clear gelatin capsule. The dissolution profile was obtained in 300 ml. of phosphate buffer or 0.1 *N* hydrochloric acid contained in an 800-ml. round-bottom dissolution flask at 37° and 60 r.p.m. with the aid of a three-blade stirrer, 4.3 cm. in diameter.

As depicted in Fig. 3, the monohydrochloride salt dissolved into phosphate buffer quite rapidly and the drug concentration in solution remained at about 0.12 mg./ml. for about 40 min. and was then followed by an apparent first-order decline of the drug concentration in solution. The amount of drug dissolved was found to be about 0.03 mg./ml. even at 2 hr. postdissolution. This finding will be investigated further. The free base dissolved quite slowly in phosphate buffer and attained only 0.02 mg./ml. at the end of 2 hr. It is apparent that the lower rate of dissolution of the free base in phosphate buffer compared to that of the monohydrochloride salt could be responsible for the failure of the free base to produce a significant *in vivo* hypotensive response in dogs when the compounds were introduced into the lumen of a loop of the small intestine.

The dissolution profiles of the encapsulated free base and the monohydrochloride salt in 0.1 *N* hydrochloric acid are also shown in Fig. 3. It was found that both the free base and the monohydrochloride salt went into solution very rapidly. The apparent initial dissolution rate was slightly greater for the free base than the monohydrochloride salt. This was in close agreement with the intrinsic dissolution rates of these two compounds found in 0.1 *N* hydrochloric acid, as clearly shown in Fig. 1.

The fact that the three salt forms of I produced a marked hypotensive effect but the corresponding free base did not points out that it is advisable to investigate routinely the effect of the salt form on the biological and pharmacological response when searching for a potential candidate of a drug substance. Compounds having the same chemical moiety but differing in the salt form may exert different degrees of biological activities, as shown in this investigation.

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