An explanation for the extended range could be that, in most cases, the factor $[b_A + b_B - b_\dagger]$ is close to zero. Both b_A and b_B can be determined experimentally by measuring activity coefficients as a function of ionic strength for the reactants A and B. Of course, $b_\dagger$ is not subject to direct experimental determination.

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In Vitro and *In Vivo* Chlorpromazine Availability from Flocculated Polysalt Complex Systems

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Abstract □ An insoluble drug–polysalt complex of chlorpromazine hydrochloride, sodium carboxymethylcellulose, and protamine sulfate was selected as a model to evaluate the effects of these macromolecular constituents on the *in vitro* and *in vivo* availability of the interacted drug. The *in vitro* liberation of drug from the polysalt complex was studied in simulated gastrointestinal fluids as a function of particle size, pH of formation of the complex flocculate, and presence and absence of enzymes in the medium. The *in vitro* drug-release studies conducted under these varying conditions suggested that the product possessed prolonged-release properties. In contrast, the *in vivo* studies with rats revealed a promoted bioavailability of the drug in the presence of the polysalt complex. Protamine sulfate, a known pinocytotic inducer, was observed to be specifically implicated in this phenomenon.

Keyphrases □ Flocculated complex systems, polysalt—chlorpromazine-^{35}S availability □ Chlorpromazine-^{35}S-polysalt complex systems—*in vivo–in vitro* availability □ Release rates—chlorpromazine-^{35}S-polysalt complex □ pH effect—chlorpromazine-^{35}S-polysalt complex stability □ Scintillometry—analysis

The physical form of a polyelectrolyte salt complex (polysalt) is a function of the charge densities of the interacting species. Macromolecules with high charge densities tend to form precipitates (flocculates), while those with lower charge densities tend to form a gel (coacervate) or quasiliquid product (1). The use of complex coacervates as a means of microencapsulating drugs has been claimed in a number of patents (2–7). Luzzi and Gerraughty (8, 9) have noted that complex coacervates of gelatin and acacia with pentobarbital may be employed to control the liberation of the encapsulated material into gastrointestinal fluids. They studied the release rate of the drug as a function of pH, temperature, ratio of solid to encapsulating material, quantity of hardening agent (formaldehyde), and addition of surfactants. In contrast, polysalt flocculates have received relatively little attention in the pharmaceutical literature.

The present drug–polysalt system was chosen on the basis of preliminary studies. It was found that a polysalt of sodium carboxymethylcellulose (NaCMC) and protamine sulfate interacted with appreciable quantities of

chlorpromazine hydrochloride (CPZ HCl) to form a drug–polysalt complex. This material was insoluble at pH 7.40 and very slowly soluble in 0.1 *N* hydrochloric acid. Another important characteristic was the presence of a proteolyzable polymer. Therefore, it was proposed that a system of this type may be of value in controlling the liberation of the interacted drug in gastrointestinal fluids by utilizing not only its slow dissolution but also the possible destruction of the flocculated drug–polysalt matrix through enzymatic degradation. The present work was directed to elucidate *in vitro* release properties of the drug–polysalt complex in simulated gastrointestinal fluids and to compare these results with the *in vivo* behavior of the system as reflected in its pharmacological response characteristics.

MATERIALS AND METHODS

Materials—All reagents were analytical grade, except barbital NF. The protamine sulfate utilized as the polycation in this work represented a highly purified protein which met all USP requirements.[1] A low-viscosity grade (25–50 cps. in 2% solution) of NaCMC[2] was employed as the polyanion. The specific activity of the ^{35}S-CPZ HCl,[3] initially 3.57 μc./mg., was adjusted to 20,000 d.p.m./mg. with unlabeled drug,[4] and this powder dilution was used in all subsequent experiments. Animals utilized in the *in vivo* portion of this study were 75-day-old albino male rats, Holtzman[5] strain.

Solutions—A pH 7.70 buffer was prepared from 0.05 *M* barbital and sufficient sodium hydroxide. Sodium acetate (0.05 *M*) and hydrochloric acid were employed to prepare a pH 4.75 buffer. Gastrointestinal fluids were prepared according to USP XVII unless otherwise noted. The scintillation cocktail XDC (10) was utilized as a counting solution. When required, approximately 15.0 ml. of the scintillation fluid was added to a low potassium glass counting vial[6] containing 5.0–5.5% colloidal silica pigment.[7] A thick gel promptly formed which was suitable for suspending any materials precipitated from solution by the XDC.

Preparation of Labeled CPZ–Polysalt Complex—Buffered stock solutions of the polymers were prepared. An aliquot of the NaCMC solution was transferred to a beaker containing an accurately weighed quantity of the ^{35}S-CPZ HCl. The mixture was stirred until a precipitate had completely formed. Protamine sulfate solution was then added with constant stirring to flocculate the system. The contents of the beaker were filtered through a medium sintered-glass funnel with the aid of suction. The gummy mass was vacuum dried to constant weight, crushed in a mortar, and assayed for drug content. Drug–polysalts were prepared in this manner from ^{35}S-CPZ HCl, 2 parts protamine sulfate, and 3 parts NaCMC. Drug–polysalt complexes flocculated in buffers at pH values of 4.75 and 7.70 were designated drug–polysalt A and drug–polysalt B, respectively. Tablets of these drug–polysalt materials, weighing approximately 250 mg. and containing approximately 100 mg. of drug, were prepared by direct compression on a Carver laboratory press with a 0.78-cm. (0.31-in.) standard cup punch and die set using an applied load of 500 lb./in.^{2}.

Preliminary work had revealed that this procedure and the order of combination of polymers in the ratio of 2 parts protamine sulfate to 3 parts NaCMC (in the presence of drug) yielded a flocculated product which was optimal with respect to the quantity of drug and colloids cleared from solution. Flocculated drug–polysalts prepared in three separate replications at each pH were found to contain 41.05 ±1.5% and 40.08 ±0.59% w/w of drug for drug–polysalt A and drug–polysalt B, respectively. In the absence of drug, maximum quantities of the polymers were found to be flocculated and cleared from solution when the polymers were combined in electrically equivalent quantities. For example, the apparent equivalent weights of protamine sulfate and NaCMC in the pH 7.70, 0.05 *M* barbital buffer were determined, from the measurement of Donnan membrane potentials, to be 1260 and 615 g. per equivalent of charge, respectively. As anticipated, the most stable flocculates were obtained when the polymers, in the absence of drug, were combined in approximately the ratio of 2 parts protamine to 1 part NaCMC.

Radioassay for ^{35}S-CPZ HCl—Solutions of the labeled drug were counted for 10 min. (or 10,000 counts) in a Packard[6] Tricarb model 574 liquid scintillation spectrometer. The counting efficiency was determined by the internal standard method. Before assay for drug content, samples of the flocculated drug–polysalt were pretreated with hot concentrated sulfuric acid to effect solution.

***In Vitro* CPZ Release Methodology**—A dissolution dialysis technique was selected for the *in vitro* drug-release studies in an attempt to simulate *in vivo* conditions. The polymer impermeable membrane utilized was intended to approximate the behavior of the gastrointestinal mucosal membrane by retaining soluble drug–polymer complexes released from the drug–polysalt system. Only the availability of the free drug, and perhaps drug associated to very small fragments of proteolyzed protamine, would be measured. Therefore, the dissolution dialysis release profile may be expected to be more closely representative of the *in vivo* absorption of CPZ from the polysalt complex than a release profile based upon dissolution alone.

Dissolution dialysis experiments utilized a cell composed of a sac of Nojax[2] cellulose tubing which contained 10 ml. of dialysis fluid and the materials to be dialyzed. Before use, the casing was boiled in 5% acetic acid and flushed well with distilled water. The sac was prepared by cutting the tubing to the required length and tying one end closed. The materials were introduced through the open end of the casing, which was then tied tightly with nylon cord at the upper level of the liquid. Free casing above the closure was knotted around the cord and the excess trimmed off. Sacs prepared in this manner had approximately the same surface area. The sacs were immersed in round, amber, 90-ml. (3-fl. oz.) bottles filled with 50 ml. of the dialysis fluid. The cap of the cell was sealed with tape. The *in vitro* release characteristics of the labeled drug–polysalts were evaluated in simulated gastric fluid, simulated intestinal fluid, and simulated intestinal fluid without enzymes. The drug-release cells were assembled and placed on the reciprocating platform of a shaker-type water bath maintained at 37°. At appropriate intervals during an 8-hr. period, samples of the (outside) fluid were removed from the cells and transferred to counting vials. An equal quantity of the test fluid was then added to the cell to maintain a constant volume. All fluids were freshly prepared and prewarmed to 37° before use. The cells were agitated at a constant rate of approximately 60, 3.8-cm. excursions/min.

***In Vivo* Studies of the Drug–Polysalt System**—The criterion selected for evaluating the *in vivo* characteristics of the flocculated drug–polysalt complex was the depression of spontaneous motor activity in rats. Williamson[8] activity cages (11), which showed negligible differences in sensitivity, were employed to measure this pharmacologic response. The ED_{50} to produce the desired effect in the rat has been reported as 4.4 mg./kg. of CPZ HCl (12). Dragstedt (13) emphasized the importance of administering identical doses of drug in the same fashion when testing products for sustained-release properties. In accordance with this principle, all animals that received a dosage form of the drug as a treatment were given 4.4 mg./kg. of CPZ HCl.

In the first set of *in vivo* experiments, rats were selected at random in groups of three at a time from a colony of 32 previously untreated animals. Each rat in the colony was fasted for 24 hr., orally dosed with polysalt blank (*i.e.*, polymers interacted in the absence of drug), and placed in the Williamson cages for 11.5 hr. The spontaneous activity of these rats was then redetermined in the same manner, except that one of the four treatments was substituted for the polysalt blank. The animals received either a second dose of polysalt blank, a 40% mixture of CPZ HCl in mannitol (drug control), drug–polysalt A, or drug–polysalt B. To optimize sensitivity, the experiments were run from 8:00 p.m. until 7:30 a.m. Rats are nocturnal animals and therefore most active at night. Influences arising from environmental disturbances were minimal

[1] The analytical data and a generous sample were kindly provided by Dr. J. M. McGuire, Eli Lilly and Co., Indianapolis, Ind.
[2] Cellosize CMC, Union Carbide Co., New York, N. Y.
[3] The Radiochemical Centre, Amersham, England.
[4] Thorazine, Smith, Kline & French Laboratories, Philadelphia, Pa.
[5] The Holtzman Co., Madison, Wis.
[6] Packard Instrument Co., Downers Grove, Ill.
[7] Cab-O-Sil, Cabot Corp., Boston, Mass.
[8] Williamson Development Co., West Concord, Mass.

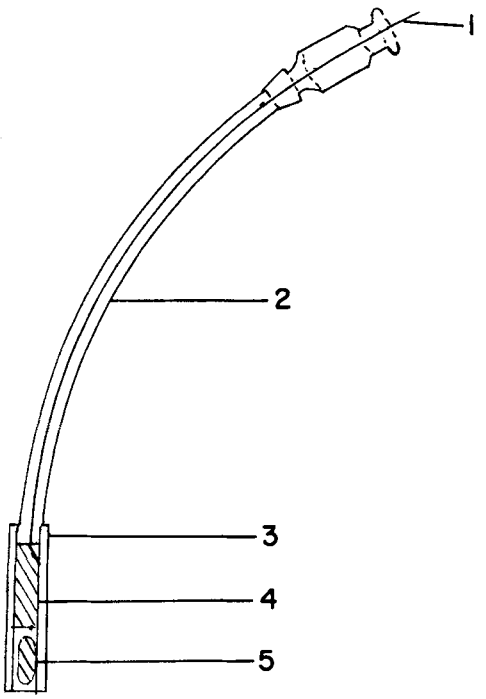

Figure 1—*Device utilized to dose rats orally for* in vivo *activity studies. Key: 1, copper wire; 2, oral dosing needle; 3, capsule holder; 4, epoxy plunger; and 5, capsule section.*

during this time period. Each of the four treatments was replicated on eight different rats.

A dosing device was developed to administer the treatments orally. The apparatus, illustrated in Fig. 1, consisted of an oral dosing needle through which a copper wire bearing a dried epoxy resin plunger was inserted at the far end. A section of catheter tubing was attached to the beveled end of the needle over the plunger. The material that the animal received was weighed into the bottom half of a tared No. 5 gelatin capsule which was subsequently moistened until flexible, folded in half, and placed in the section of tubing. The dosing needle was then inserted into the esophagus of the animal; the copper wire plunger rod was pushed forward to eject the capsule. Since rodents do not have the ability to regurgitate, the capsule and its contents were carried to the stomach by peristalsis. Immediately following dosing with the capsule, the animal received about 0.5 ml. water through the dosing needle.

A second set of *in vivo* experiments was conducted on a new colony of 18 rats according to the general procedure already outlined. The three treatment groups were a drug control of 40% CPZ HCl in mannitol, a physical mixture of 40% CPZ HCl and protamine sulfate, and particles of drug–polysalt which would not pass a 30-mesh screen. Each of the three treatments was replicated on six different rats.

RESULTS AND DISCUSSION

***In Vitro* Release Studies in Simulated Gastric Fluid**—Figure 2 illustrates the dissolution dialysis of various physical forms of the drug in simulated gastric fluid. The uppermost curve in Fig. 2 was obtained with 100 mg. of ^{35}S-CPZ dissolved in 10 ml. of simulated gastric fluid contained within the dialysis sac. This is the same quantity of CPZ as was contained in the drug–polysalts studied in the same manner. However, the dialysis sac served in this case to restrain the ready transfer of CPZ into the outside compartment. It is apparent from the other curves that the presence of a polysalt complex provided a significant additional retardation.

Comparison of the curves for the tablets prepared from drug–polysalt B and small particles of the same material (>30 mesh) reveals the delaying effect of compression on the release of CPZ. This result obviously implicates the surface area of the drug–polysalt as a factor affecting the release of drug from the system.

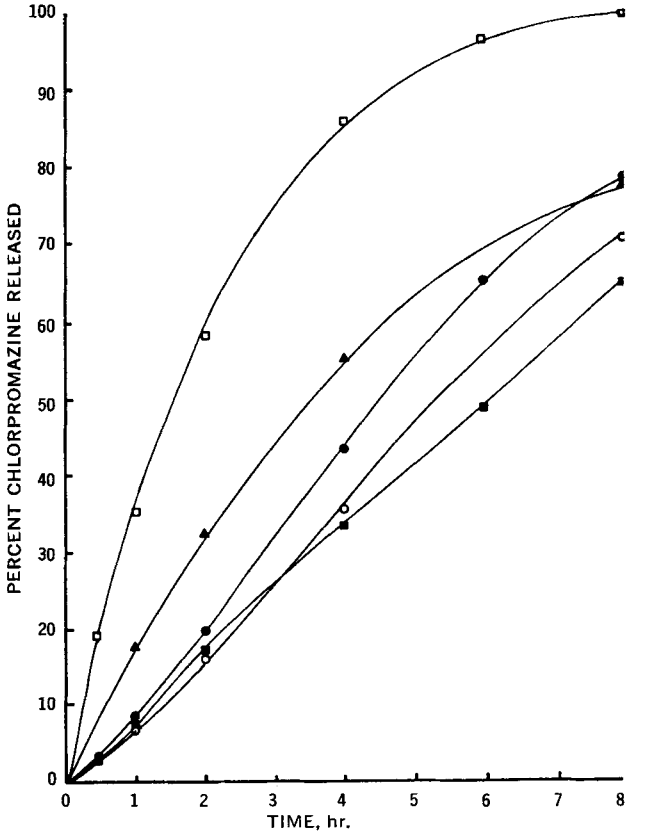

Figure 2—In vitro *release of ^{35}S-labeled chlorpromazine through cellulose sacs into simulated gastric fluid. Key:* □, *dialysis solution control of drug alone;* ●, *tablets drug–polysalt A;* ■, *tablets drug–polysalt B;* ▲, *particles drug–polysalt B (>30 mesh); and* ○, *tablet control (physical mixture of polymer and drug).*

Upon immersion of the drug–polysalt tablets in gastric fluid, the cream-white biconcave disk appeared to swell slightly; a uniform three-dimensional gray band began to form around the outer edge, widen, and move gradually toward the core. After 8–10 hr., the cream-white solid matrix was replaced by a gray, swollen, gel-like structure. From these visual observations, it can be hypothesized that as the simulated gastric fluid penetrated the matrix, it began to solubilize the protamine, forming a network of channels in the

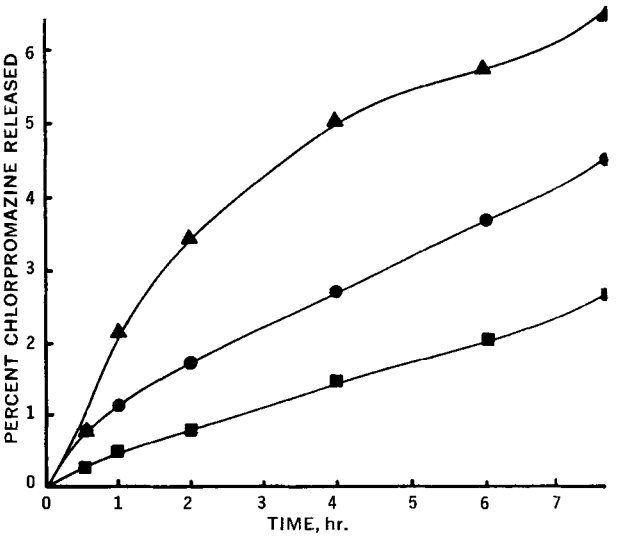

Figure 3—*Release of ^{35}S-labeled chlorpromazine from drug–polysalt tablets into simulated intestinal fluid without enzymes. Key:* ▲, *tablet control (physical mixture of polymers and drugs);* ■, *tablets drug–polysalt A; and* ●, *tablets drug–polysalt B.*

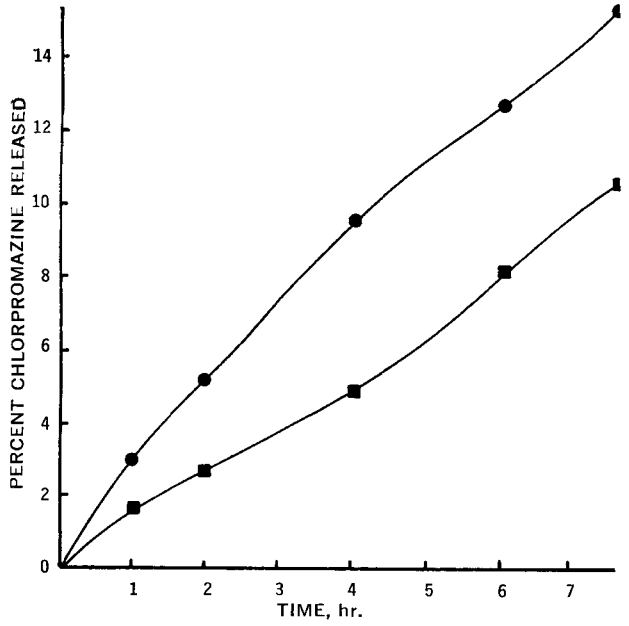

Figure 4—*Release of ^{35}S-labeled drug–polysalt tablets through cellulose membrane into simulated intestinal fluid with enzymes after immersion in simulated gastric fluid for 2 hr. Key: ●, tablets drug–polysalt A; and ■, tablets drug–polysalt B.*

remaining structure of insoluble free acid CMC. The entrapped drug was also solubilized by the gastric fluid; as channels were created, it dissolved and slowly leeched out into the surrounding medium.

CPZ Release in Simulated Intestinal Fluids—At pH 7.4 the tablets swelled rapidly and the matrix soon broke apart into large cream-white particles. The particles, although swollen, appeared to retain their structural integrity in contrast to the behavior of the polysalt complex in gastric fluid. Figure 3 illustrates the slow release of drug from the polysalt system in simulated intestinal fluid without enzymes. Figure 4 presents the dissolution dialysis profile of drug–polysalt A and B tablets in simulated intestinal fluid with enzymes following pretreatment by immersion, for 2 hr., in gastric fluid. This system was intended to simulate *in vivo* conditions where the enzymes can serve as additional means of degrading the complex and liberating the drug. However, in all cases with intestinal fluid, only relatively small amounts of CPZ were released. This can be attributed to the insolubility of the drug and the stability of its polysalt matrix in this medium. It was found that a control study of CPZ dialysis could not be performed for intestinal fluid in the same manner as that done for gastric fluid because of the limited solubility of the drug in this medium.

Influence of pH of Formation on the Stability of the Drug–Polysalt Complex—Since both the drug and polymers are weak electrolytes, the extent of drug–polymer and polymer–polymer electrostatic interactions would be expected to vary with pH. A given drug–polysalt complex should be maximally stable at its pH of formation when combined in electrically equivalent quantities. It is, therefore, also expected that the complex formed at pH 4.75 would be more stable in solutions of lower pH than a corresponding product flocculated at pH 7.70. Conversely, the drug–polysalt complex formed at pH 7.70 should be more stable in solutions at higher pH values relative to the product flocculated at pH 4.75. The release profiles of drug–polysalt complex tablets A and B (shown in Fig. 2) are clearly inconsistent with such expectations. Figure 2 reveals a faster and more complete liberation of CPZ in acid media from the drug–polysalt A tablet than from its B counterpart. Furthermore, visual observations during the study indicated that the tablet matrix of drug–polysalt A was less cohesive than that of the corresponding B product. The observed phenomenon is partly explicable in terms of the greater solubility of CPZ salt relative to free base. In the pH 7.70 formed drug–polysalt complex, the CPZ occurs to a greater extent in the less soluble form of the free base relative to the drug in the pH 4.75 formed complex. Although the relatively slow dissolution of the unionized form of the drug is a factor contributing to the limited release rate of the drug from the tablet matrix, it is not wholly responsible for the slow release. This is indicated in Fig. 3 by the observed faster drug release from a physical mixture of the drug with the polymers relative to the flocculate where, neglecting the occurrence of localized drug–polymer interaction, the rate-limiting factor in the availability of the drug from the tablet may be presumed to be its dissolution in the medium. The similarity in the

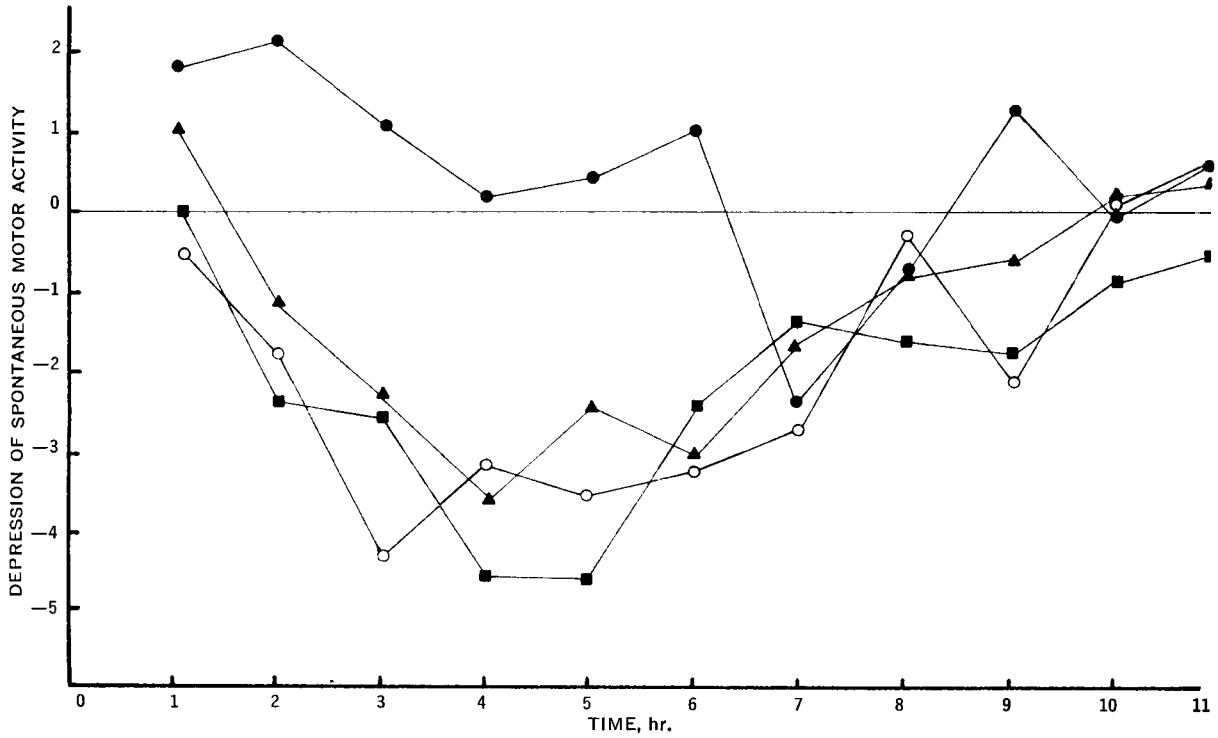

Figure 5—*Chlorpromazine-induced depression of spontaneous motor activity of rats as a function of time following administration of chlorpromazine in different dosage forms. Each point is the average of eight replications on different animals. Key: ●, polysalt blank; ▲, drug control (chlorpromazine HCl and mannitol); ■, drug–polysalt A particles; and ○, drug–polysalt B particles.*

Table I—Summary of Observed Variance Ratio ($F_{obs.}$) Showing Values for Significant Differences among the Four Treatment[a] Means

Time, hr.	Mean Square Treatments	Mean Square Error	$F_{obs.}$ (3, 28)[b]
1	8.948	7.388	1.210
2	31.780	2.000	15.890[c]
3	40.370	7.280	5.540[c]
4	35.783	6.861	5.520[c]
5	38.700	5.250	7.234[c]
6	33.083	9.438	3.505[d]
7	3.533	6.370	0.555
8	7.283	6.191	1.176
9	17.917	4.938	3.628[d]
10	2.083	6.045	0.345
11	2.707	7.063	0.383

[a] The four treatments referred to the polysalt blank, drug control, drug–polysalt A, and drug–polysalt B. [b] $F_{0.95}$ (3, 28) = 2.95; $F_{0.99}$ (3, 28) = 4.57. [c] Significant at $p < 0.01$. [d] Significant at $p < 0.05$.

curves shown in Fig. 2 for drug release from tablets composed of a physical mixture and drug–polysalt B indicates, however, that under acidic conditions in the medium the slow dissolution of the unionized drug may be an important factor in limiting the drug's release.

Another factor may be one similar to the electrolyte plasticization effect observed by Michaels (14); the ions constituting the salt form of the drug can act as a shield to decrease the electrostatic attraction between polycation and polyanion, resulting in a less consolidated system with a greater elasticity and tendency to swell. The looser polymer network formed at pH 4.75 by this mechanism could allow the physically entrapped drug to be more readily released from the matrix.

Proposed Mechanism of Drug–Polysalt Interaction—Based on the preliminary observations and *in vitro* release studies, it can be hypothesized that the mechanism of uptake of the drug by the polysalt involves two consecutive processes. The drug first becomes bound to the NaCMC; upon addition of the protamine polycation, which possesses a greater affinity for the anionic binding sites on the CMC, some bound CPZ is competitively displaced. The released drug may then precipitate from solution due to local saturation and become physically entrapped within the solid polysalt matrix. The drug, therefore, likely exists in both a mechanically entrapped and physically adsorbed form within the polysalt matrix. Its mechanisms of release accordingly include desorption and dissolution.

***In Vivo* Studies of CPZ Availability**—CPZ is cleared very rapidly from the bloodstream (15); the time course of pharmacological response intensity, *i.e.*, magnitude of depression of spontaneous motor activity, was studied in rats in lieu of direct determinations of the drug in body fluids. Male rats were selected because their general level of activity, although lower than the female's, is not subject to the cyclization accompanying the female's estrus cycle. The results of the first study are illustrated in Fig. 5. The "deviation" statistic utilized to describe the treatment-induced effects was obtained as the difference in pretreatment and posttreatment activity readings. The mean of this value for each treatment group was plotted as a function of time. A single factor analysis of variance was employed to indicate the presence of an overall significant difference among the four treatment groups at each hourly interval. At those time periods where the variance ratio, $F_{obs.}$, exceeded the critical value for the 0.05 probability level, comparison tests were employed to determine the exact group(s) responsible. These tests are summarized in Tables I and II. Table I is related to Fig. 5 in that it indicates the times and probability levels at which treatment-induced effects are significant. Table II specifies which treatments are responsible. It is obvious from the results that, contrary to expectation, no prolonged action effect was demonstrated by the drug–polysalt complexes.

However, a closer inspection of the results over a 6-hr. period following the treatments revealed that the depression of activity in one or both of the groups receiving a drug–polysalt was consistently greater than that in the group receiving the drug control. A comparison test, utilizing the average deviation of the two drug–polysalt treatment groups *versus* the drug control group, revealed a statistical difference in activity for the 6-hr. period at approximately the 0.15 probability level.

Since only a heterogeneous mixture of drug–polysalt particles was utilized in the first experiment, it was felt that the use of larger particles (>30 mesh) might provide the desired sustained action. (The small quantity of drug–polysalt complex administered precluded its direct compression into tablets.) Of equal interest was an investigation into the possible cause of the apparently enhanced activity of the drug. The ability of such proteinaceous materials as protamine sulfate to act as inducers of pinocytosis has been well documented in the literature (16–21). Indeed, the striking observations of Whitmore (23), that gelatin microcapsules of streptomycin exhibited enhanced transport into polymorphonuclear leukocytes and macrophages, take on new meaning when he attributes the phenomenon to "phagocytosis" (*sic*) and cites as evidence the high degree of cytoplasmic vacuolation. These facts could conceivably provide a basis on which to explain the enhanced drug activity. Hence, another treatment variable was introduced to determine the effect of protamine sulfate alone on the biological availability of CPZ. Based on these considerations, a second set of *in vivo* experiments was performed. The results are shown in Fig. 6. A single factor analysis of variance (22) for significant differences among the various treatment combinations over an 11.5-hr. period is summarized in Table III. These data clearly demonstrate that the depression of spontaneous activity produced by both treatments was greater than that of the drug control group. Despite the larger particles used, however, no significant sustained action of the drug–polysalt complex was observed. There are several possible explanations for these findings. It is apparent that the presence of protamine alone and with CMC enhances the activity of CPZ. The mechanism by which this occurs is probably not merely Donnan exclusion (24), since drug release *in vitro*, where this nonspecific effect would also be manifested for the interacted system, is delayed rather than facilitated. Although the results of the present study do not provide direct evidence for its occurrence, stimulation of pinocytosis or some other effect of the polymers on the mucosal-absorbing surface in the gastrointestinal tract appears to be a reasonable possibility. If protamine does have the ability to induce cells of the gastrointestinal mucosal-absorbing surface to envacuolate the surrounding medium, it could enhance the absorption of any drug present in that medium. If this was shown to be the case unequivocally, an entirely new concept of drug dosage design could be initiated. However, with the drug–polysalt system, there may be additional components to the mechanism involved in the augmented activity of the drug. Because CPZ is unstable in acidic solution, the polysalt complex possibly also acts to protect the drug from destruction in the stomach and thereby allows a larger fraction of the released medicament to be absorbed intact. Still another

Table II—Comparison Tests at Intervals Showing Significant Differences[a]

Time, hr.	Treatments Compared[b]	$F_{obs.}$ (3, 28)[c]
2	1 × 2	21.13[d]
	1 × 3	38.28[d]
	1 × 4	32.00[d]
3	1 × 2	6.26[d]
	1 × 3	7.22[e]
	1 × 4	15.87[d]
4	1 × 2	8.75[d]
	1 × 3	13.86[d]
	1 × 4	7.66[d]
5	1 × 2	6.73[e]
	1 × 3	19.64[d]
	1 × 4	11.96[d]
6	1 × 2	7.21[e]
	1 × 3	5.19[e]
	1 × 4	8.11[e]
9	1 × 2	2.48
	1 × 3	6.70[e]
	1 × 4	9.23[d]

[a] 2 × 3, 2 × 4, 3 × 4 Comparisons not significant. [b] 1 = polysalt blank, 2 = drug control, 3 = drug–polysalt A, 4 = drug–polysalt B. [c] $F_{0.95}$ (1, 28) = 4.20; $F_{0.99}$ (1, 28) = 7.64. [d] Significant at $p < 0.01$. [e] Significant at $p < 0.05$.

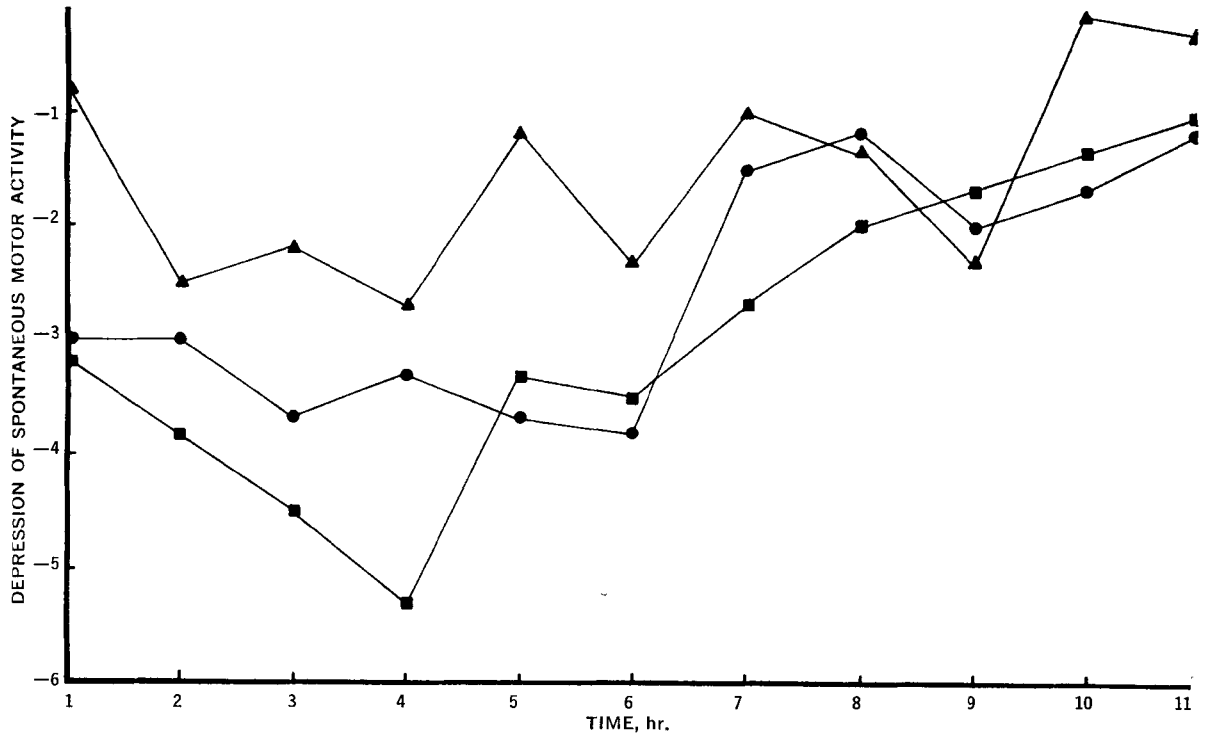

Figure 6—*Chlorpromazine-induced depression of spontaneous motor activity of rats as a function of time following administration of chlorpromazine in different dosage forms. Each point represents the average of six replications on different animals. Key:* ▲, *drug control (chlorpromazine HCl and mannitol);* ●, *chlorpromazine hydrochloride–protamine sulfate physical mixture; and* ■, *chlorpromazine–polysalt particles (>30 mesh).*

alternative possibility in explaining the phenomena is that the drug is released from the system as a protamine complex which is absorbed more rapidly. The CMC may also be implicated in this process, since it can be taken up by mammalian tissue (25); indeed, CPZ itself has been reported to affect absorption through cell membranes (26). Thus, it may even be speculated that the particular combination of materials in the interacted system possesses a maximal ability to affect cellular uptake and enhance the pharmacologic response to CPZ.

The conditions employed in the *in vitro* release studies are obviously only a crude approximation of those actually obtained *in vivo*. Consequently, a diversity of factors may have been operative to produce the clearly evidenced lack of correlation between the *in vitro* and *in vivo* drug availability. Notable among these factors are the small volumes of gastrointestinal fluid available for the dissolution of the drug *in vivo* relative to that used in the *in vitro* study, as well as differences in mechanical agitation of the drug-releasing materials. These differences could contribute significantly to the discrepancy between the *in vitro* and *in vivo* results. Conceivably, *in vivo* the drug-release medium could have become rapidly saturated with drug. This would result in the passage of the drug across the gastrointestinal barrier to become the rate-limiting step in its absorption rather than its release from the dosage form. In this case, the differences in *in vivo* drug availability noted for the different formulations could be attributed to the specific effects of the macromolecular constituents of the dosage form on the barrier properties of the absorbing surfaces and/or their effects on the stability of the drug in the fluids. Because of the demonstrated ability of such materials in altering the permeability properties of cells, the former effect may be considered more likely.

Also, in considering the relatively small volumes of fluid available for action upon the administered materials *in vivo*, it cannot be neglected that gelatin-encapsulated particles were used *in vivo* while compressed tablets were used *in vitro*. The use of gelatin-encapsulated particles was necessitated by the difficulties in compressing the small amounts of drug–polysalt complex comprising the dose of CPZ for the rats. A slow dissolution of the gelatin capsules in the limited fluids available *in vivo* could have slowed the release of the drug in all cases and also masked the differences among the formulations such as were observed *in vitro*.

Table III—Results of Single Factor Analysis of Variance of Treatment Combinations for an 11.5-hr. Period

Treatments[a]	Mean Square Treatments	Mean Square Error	$F_{obs.}$ (1, 10)[b]
1 × 2	330.73	59.43	5.57[c]
1 × 3	705.34	110.03	6.41[c]

[a] 1 = drug control, 2 = protamine sulfate–chlorpromazine hydrochloride physical mixture, 3 = drug–polysalt particles. [b] $F_{0.95}$ (1, 10) = 4.96; $F_{0.99}$ (1, 10) = 10.0. [c] Significant at $p < 0.05$.

CONCLUSIONS

In vitro dissolution dialysis studies clearly indicated a prolongation of drug release from polysalt flocculates. The *in vivo* results with rats did not, however, support this conclusion. Nevertheless, these results do elicit the speculation that, should the observed *in vivo* effects of the polymeric constituents on CPZ availability be a consequence of an active alteration of the permeability properties of the mucosal barrier, *e.g.*, endocytotic induction, it may also be possible, in general, to promote similarly the absorption of irregularly and poorly absorbed orally administered drugs.

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Cytochrome P-450 and Alkaloid Synthesis in *Claviceps purpurea*

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Abstract □ Tryptophan, 4-dimethylallyltryptophan, and various analogs of tryptophan were investigated for their effect on binding to cytochrome P-450 and on cytochrome P-450 and total alkaloid levels in *Claviceps purpurea*. These compounds were shown not to affect cytochrome P-450 levels, in contrast to phenobarbital which increased the levels, but rather exhibited stereospecific binding to cytochrome P-450. Those compounds (L-tryptophan and L-4-dimethylallyltryptophan), which showed the highest binding affinity and are known precursors, caused the greatest increase in total alkaloid levels. The significance of these findings is discussed.

Keyphrases □ Cytochrome P-450, alkaloid synthesis—*Claviceps purpurea* □ ^{14}C-L-Tryptophan, tryptophan analogs—cytochrome P-450 □ *Claviceps purpurea* growth—tryptophan analogs □ Colorimetric analysis—spectrophotometer

The occurrence of cytochrome P-450 in a clavine-producing strain of *Claviceps purpurea* has been previously reported (1). Phenobarbital-treated *C. purpurea* exhibited a parallel increase in the cytochrome P-450 and in total alkaloid. Cyanide produced a gradual but marked decrease in both cytochrome P-450 and alkaloid levels. The interconversion of cytochrome P-450 and P-420 has been utilized to provide additional evidence for its presence in *C. purpurea* (2).

Cytochrome P-450 is now recognized as an "oxygen-activating enzyme," participating in the hydroxylation of a wide variety of compounds (3, 4). Substrate interaction with cytochrome P-450 gives rise to two types of difference spectra which have been utilized to measure the nature and degree of interaction of cytochrome P-450 with various compounds. Type I spectral changes are characterized by a difference spectrum, with a trough at 420 mμ and a peak at 385 mμ, and are characteristic of that produced by the interaction of cytochrome P-450 with hexobarbital, aminopyrine, phenobarbital, and chlorpromazine. Type II spectral changes exhibit a difference spectrum, having a peak at 430 mμ and a trough at 390 mμ; the spectrum is produced by the interaction of aniline, nicotine, nicotinamide, *etc.*, with cytochrome P-450. The magnitude of the spectral changes is substrate concentration-dependent (5). It has been suggested (6) that the substrate forms complexes with the oxidized form of cytochrome P-450 and that the rate-limiting step of the reaction is the reduction of the substrate–cytochrome P-450 complex. However, it has also been suggested that the complex is more readily reduced by NADPH cytochrome P-450 reductase than is cytochrome P-450 in the absence of the substrate (7).

The present study is concerned with the possible role of cytochrome P-450 in alkaloid synthesis and extends the previously reported data. The binding of tryptophan, tryptophan analogs, isopentenyl pyrophosphate, and 4-dimethylallyltryptophan to cytochrome P-450 was studied using the difference absorbance. The effect of these compounds on the levels of cytochrome P-450 and total alkaloid and the correlation of these values with effects on binding are also reported.

EXPERIMENTAL

Binding of ^{14}C-L-Tryptophan to Cytochrome P-450—The method utilized was similar to that of Orrenius and Ernster (8) who studied