Complexes of Ergot Alkaloids and Derivatives II: Interaction of Dihydroergotoxine with Certain Xanthines

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Abstract □ Intermolecular complexation between a mixture of the comparatively water-insoluble alkaloids comprising 9,10-dihydroergotoxine and several xanthines was investigated. Substantial elevations of aqueous solubility of dihydroergotoxine in 0.1 *N* HCl and in pH 6.65 phosphate buffer was observed in most of the cases examined. This incongruity in the normally expected solubility data may be attributed to a mutual influence between the ergot derivative and the xanthine under consideration. Dissolution studies indicated a generally enhanced first-order rate of solution in the presence of xanthine which seems to evidence some "driving force" pulling the drug into solution. Partitioning rates (aqueous to chloroform) are usually increased when xanthine is added to the 9,10-dihydroergotoxine at pH 6.65 and the reverse is true in 0.1 *N* HCl. Biological data in cats and humans are in good agreement with physicochemical work.

Keyphrases □ Ergot alkaloids, derivatives—complex formation □ Dihydroergotoxine-xanthines—interactions □ Xanthines effect—dihydroergotoxine dissolution □ Partitioning rate, dihydroergotoxine—xanthine effect □ Biological activity correlation—physicochemical data

Caffeine and other naturally occurring xanthines have previously been shown to exhibit exceptional ability for formation of stable complexes with a wide variety of substances (1–3).

Mixtures of ergot alkaloids themselves have been stated to possess a tendency toward facile intermolecular complexation (4).

The interaction of caffeine and ergotamine tartrate in aqueous solution was observed in this laboratory and is exemplified by: (*a*) an increased solubility of the alkaloid in the presence of caffeine; (*b*) an increased dissolution rate constant for the ergot alkaloid in the combination over pure ergotamine; and (*c*) increased or decreased partitioning rates (aqueous to chloroform) of the alkaloid in the presence of caffeine relative to ergotamine tartrate alone depending on the pH studied (5).

This attraction between ergotamine tartrate and caffeine appeared to be an extremely weak one. The isolation of the complex eluded the authors (5). These ill-defined forces may be on the order of those found in dipole–dipole, dipole–induced dipole, and induced dipole–induced dipole systems, but have not been delineated. The two component solubility curves do not lend themselves to stoichiometric analysis (5).

It was speculated at the time that these *in vitro* results might correlate to some extent with the stated clinical effectiveness (6, 7) of orally administered caffeine, ergotamine tablets,[1] and the caffeine present was functioning by holding the ergotamine in solution in the gut. Consequently, this solubilization of the complex in enteral media was thought probably to provide increased drug activity through ease of absorption (5). If the above hypothesis were correct, it was hoped that judicious selection of new potential complexing agents and their *in vitro* study and comparison might lead to more potent and efficacious substances. In the light of presently available animal and human data, this theory has been corroborated.

An investigation was carried out on the naturally occurring xanthines, caffeine, theophylline, and theobromine, as well as several of their closely related and commercially available analogs in an endeavor to discover a more suitable solubilizing agent for ergotamine tartrate and other medicinally active bases derived from ergot. The ergot alkaloid under consideration in this work was 9,10-dihydroergotoxine.[2] This substance is an approximately equimolar mixture of the methanesulfonate salt of three alkaloids: dihydroergocristine, dihydroergokryptine, and dihydroergocornine. These three compounds are difficult to separate and the salts thereof display only slight water solubility, although they are not as insoluble as ergotamine. The three are closely related structurally, the only deviation being in amino acid composition of the peptide side chain attached to the lysergic acid nucleus.

The simplest basic structural requirements for this specific type of complexing agent were thought to be: (*a*) a planar ring system or single ring; (*b*) absence of acid or basic functions (the comparative electrical neutrality and lack of acidic hydrogen); and (*c*) some degree of water solubility. This premise has shown itself to be generally correct. Pharmacologically speaking, an innocuous substance is demanded. Caffeine, theophylline, and theobromine present major drawbacks as is evidenced by their own pharmacology represented by their important clinical applications.

In most of the compounds surveyed in this project the basic xanthine prototype was maintained, and it was believed that specific structural modification of this moiety might lead to more appropriate compounds. Commercially available materials were examined with the exception of trimethyl-isocyanurate ester which was prepared (8). This is not to imply that many other molecules should not possess the ability of complexation with ergot alkaloids as many systems fit the stipulations presented in the previous paragraph.

BIOLOGICAL

The onset and duration of action upon enteral administration of dihydroergotoxine and other ergot alkaloids were recently reported to be enhanced by several of the xanthine derivatives mentioned in this study. The pharmacological parameter employed was α-adrenergic blocking activity of ergot alkaloids in cats. The data clearly show the increased activity of some ergot alkaloids when used together with suitable xanthine derivatives. This appears

[1] Cafergot, Sandoz Pharmaceuticals, Hanover, N. J.

[2] Hydergine, Sandoz Pharmaceuticals, Hanover, N. J.

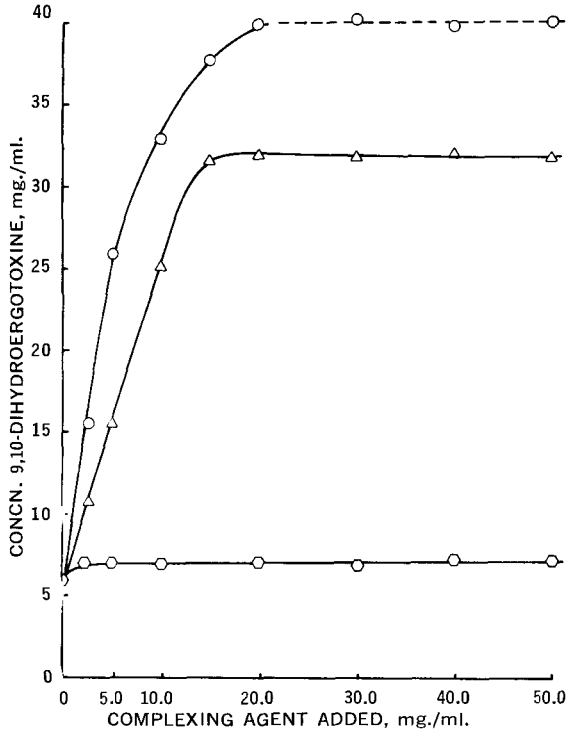

Figure 1—*Solubilizing action of xanthines on 9,10-dihydroergotoxine in 0.1* N *HCl (pH 1.25) at 30° for 24 hr. Key:* ○, *caffeine;* ○- - -, *complete solution;* △, *theophylline;* ○, *theobromine.*

to point toward the fact that this phenomenon is due to an improved absorption picture for these compounds in cats (9).

In humans the oral absorption of tritiated dihydroergocristine—one of the three alkaloidal components of dihydroergotoxine—was both faster and more complete for a 24-hr. period on administration with 7-β-hydroxypropyltheophylline. Upon the utilization in combination with the theophylline analog this was evidenced by appreciably higher and longer lasting blood levels when the xanthine

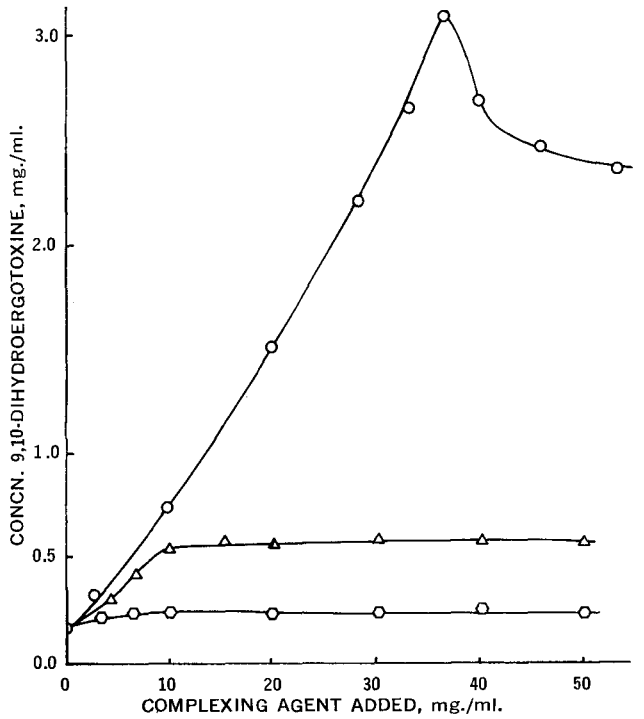

Figure 2—*Solubilizing action of xanthines on 9,10-dihydroergotoxine in phosphate buffer (pH 6.65.; ionic strength 0.2) at 30° for 24 hr. Key:* ○, *caffeine;* △, *theophylline;* ○, *theobromine.*

was present compared to when the drug alone was given. It was concomitantly noted that tritiated dihydroergocristine exhibited a significant increase in the cumulative urinary excretion of tritium when the alkaloid was administered in combination with 7-β-hydroxypropyltheophylline relative to dihydroergocristine itself (9).

EXPERIMENTAL

Materials—Dihydroergotoxine substance[2] is a mixture of equal parts by weight of dihydroergocristine methanesulfonate, dihydroergokryptine methanesulfonate, and dihydroergocornine methanesulfonate, which melted with decomposition at 196–206°. The various xanthines employed were obtained from the following sources: theophylline, m.p. 272–274° (Matheson, Coleman & Bell); theobromine (Mallinckrodt Chemical Works); caffeine anhydrous powder USP, m.p. 238° (Pfizer); 7-β-hydroxypropyltheophylline, m.p. 135–138° (Gane's Chemical Works, Inc.); 7-(2,3-dihydroxypropyl)-theophylline, m.p. 163–166° (Aldrich Chemical Co.). Melting points are uncorrected. Reagent grade chloroform (Mallinckrodt) was employed in the partitioning studies, and 0.1 *N* HCl and pH 6.65 phosphate buffer were prepared in the usual manner. All pH values were measured on a Metrhom pH meter and spectrophotometric data obtained from a Cary model 14 spectrophotometer.

Solubility Studies—Watertight screw-capped vials (18 ml.) containing exactly 10 ml. of solvent, dihydroergotoxine (100 mg. with pH 6.65 buffer and 400 mg. with 0.1 *N* HCl) and varying amounts of the xanthine, were clamped onto the edge of metal disks (15-cm. diameter) mounted on a motor-driven shaft and rotated vertically at 6 r.p.m. in a constant-temperature bath at 30 ± 0.1°. After exactly 24 hr., samples were withdrawn using pipet with filters attached and analyzed for dihydroergotoxine by the Van Urk method (10).

Dissolution Rates—Either a 25 or 60 r.p.m. stirrer motor fitted with a 2.54-cm. propeller blade placed 4 cm. from the bottom of an 800-ml. beaker containing 500 ml. 0.1 *N* HCl solution was used in determining these rates. The ergot alkaloid (50 mg.) or alkaloid in combination with xanthine (50 mg.:5 g.) was placed into the stirred solution from a height of about 1.5 cm. The temperature of

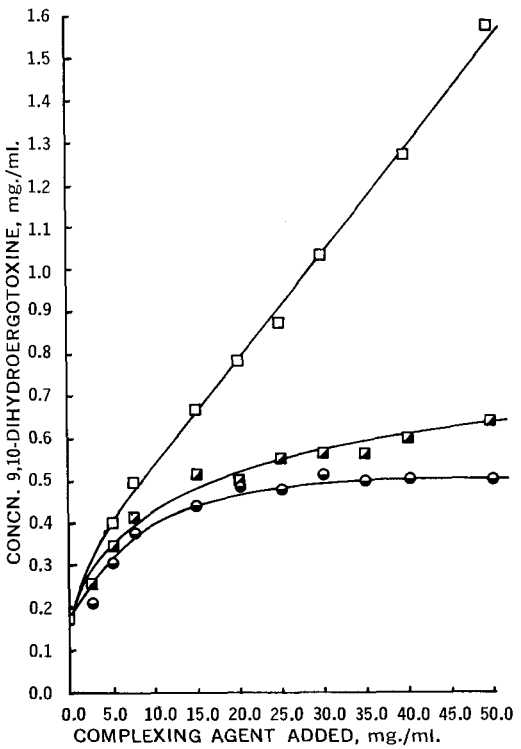

Figure 3—*Solubilizing action of compounds on 9,10-dihydroergotoxine (100 mg.) in phosphate buffer (10 ml.; pH 6.75; ionic strength 0.2) at 30° for 24 hr. Key:* ◒, *trimethyl-isocyanurate ester;* ◪, *7-β-hydroxypropyltheophylline;* □, *7-(2,3-dihydroxypropyl)-theophylline.*

the dissolution rate media was kept at 37 ± 0.1° by immersing the beaker in a constant-temperature bath. Samples were withdrawn for analysis for dihydroergotoxine content by the Van Urk method (10).

Partitioning Studies—Fifteen milliliters of an aqueous phase (0.1 *N* HCl or phosphate buffer, pH 6.65 ionic strength of 0.20) containing alkaloid or alkaloid and xanthine (1:100) made by dissolution of dihydroergotoxine, Van Urk analysis, and addition of xanthine was added carefully to 15 ml. chloroform in screw-capped vials. The vials were sealed and rotated at 6 r.p.m. in a 30 ± 0.1° constant-temperature bath using the same apparatus as described for solubility studies. Five-milliliter samples were taken at 1, 3, 5, 7, and 9-min. intervals from the aqueous phase and analyzed for alkaloidal content by the Van Urk method (10).

RESULTS AND DISCUSSION

The solubility of 9,10-dihydroergotoxine methanesulfonate varies with hydrogen ion concentration as can be seen from Figs. 1 and 2. Changes in apparent solubility of the alkaloid when caffeine is added are also a function of pH up to the point of complete solution in 0.1 *N* HCl and the downward trend evidenced in Fig. 2 (pH 6.65, phosphate buffer) after addition of 35 mg./ml. caffeine. This latter phenomenon is possibly the result of saturation of the solution with the solubilized species. It is evident from Fig. 2 that solubility of the ergot derivative is raised twentyfold through interaction with caffeine at near neutral pH and 30°. The ratio of caffeine to alkaloid which can accomplish this increase is about 10:1 on a weight basis and the molar ratio is 35:1 which brings about maximum solubilization at pH 6.65. Theophylline shows a great deal less complexation in the phosphate buffer. The plateau in Fig. 2 is conceivably caused by solubility limitations of theophylline; however, the picture in 0.1 *N* HCl generally parallels caffeine. Theobromine does not appear to form a complex as may be noted in Figs. 1 and 2. This is likely a result of the poor solubility of theobromine, about 1/40 that of caffeine.

Figure 3 illustrates the interaction of three synthetic compounds and caffeine with 9,10-dihydroergotoxine methanesulfonate in phosphate buffer. Two theophylline derivatives, 7-(2,3-dihydroxypropyl)-theophylline and 7-β-hydroxypropyltheophylline, were employed, with each exhibiting comparable solubilizing properties to caffeine. Both of these compounds have good water solubilities relative to the other xanthines. The dihydroxy derivative elevates the 9,10-dihydroergotoxine methanesulfonate solubility up to a maximum of 1.5 mg./ml. under the conditions employed, while the 7-β-hydroxy derivative gives somewhat less solubilization. Trimethyl-isocyanurate ester (1,3,5-trimethyl-2,4,6-trioxo-1,3,5-triazine) (8) showed the least interaction. This may be because of its low water solubility in addition to absence of the second ring as is found in all xanthines followed by the consequent lowering of its affinity for the ergot alkaloid.

From Fig. 2 it seems the presence of less xanthine molecules is needed to solubilize each molecule of the ergot compound than was the situation with ergotamine tartrate (5).

The question as to the species comprising the complex as well as its stoichiometry is in doubt, but protonation of one or both species is likely in 0.1 *N* HCl, while at pH 6.65 almost all the caffeine and a good percentage of the dihydroergotoxine (pKa around 6.5) is present as the neutral molecule.

The exact nature of the complex between these ergot alkaloids possessing the unusual cyclic tripeptide attached to the lysergic acid portion has not been elucidated, but work is continuing along these lines.

Complete solubility studies were not carried out in 0.1 *N* HCl as most of the alkaloid and some of the xanthine would exist as the hydrochloride salt under these conditions, and even though the amount in solution is considerable, absorption of these ionic species is doubtful (11).

The dissolution rate of poorly water-soluble drugs is considered by some to be the rate-limiting step in absorption (12–14). Dissolution rates were run on 9,10-dihydroergotoxine methanesulfonate with and without xanthine present to determine if there were any differences in rate constants. These tests were done in a relatively large volume of 0.1 *N* HCl (500 ml.) to effect "sink" conditions and allow approximation of a first-order process. A 100:1 ratio of xanthine to alkaloid was employed to correspond to the quantities found in the commercial product (6). Figure 4 gives an example of a

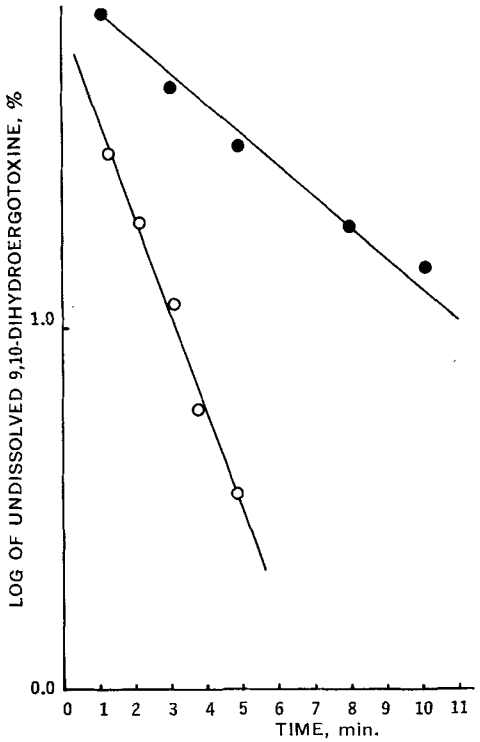

Figure 4—*Effect of caffeine on the dissolution rate of 9,10-dihydroergotoxine in 0.1* N *HCl (500 ml.) at 37° and 60 r.p.m. Key:* ●, *dissolution of 9,10-dihydroergotoxine, 50 mg.;* ○, *dissolution of 9,10-dihydroergotoxine, 50 mg., and caffeine, 5.0 g.*

system of this kind and substantial variation may be noted in the two rate constants (a factor of three). The sizable variances in the relative rate constants are again evidenced in Fig. 5 with the ex-

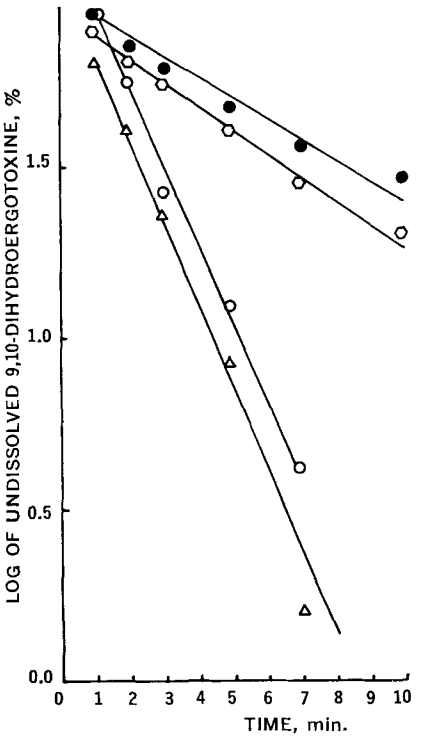

Figure 5—*Effect of naturally occurring xanthines on the dissolution rate of 9,10-dihydroergotoxine in 0.1* N *HCl (500 ml.) at 37°, stirring speed 25 r.p.m. Key:* ●, *50 mg. dihydroergotoxine;* ○, *50 mg. dihydroergotoxine and caffeine, 5.0 g.;* △, *50 mg. dihydroergotoxine and theophylline, 5.0 g.;* ○, *50 mg. dihydroergotoxine and theobromine, 5.0 g.*

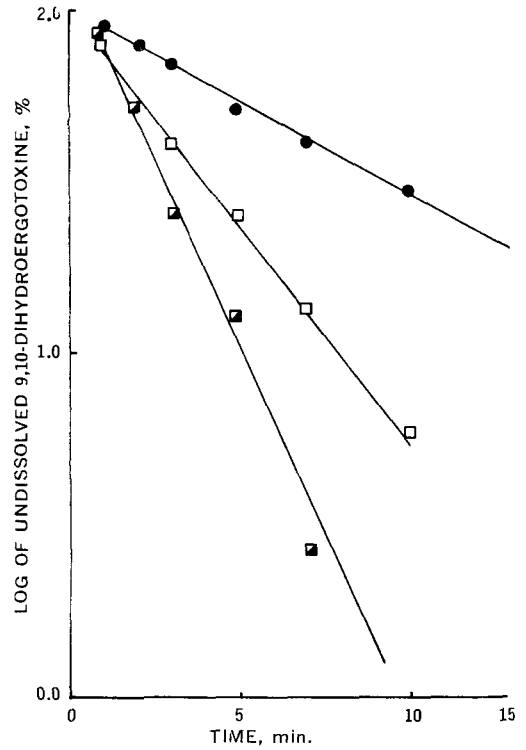

Figure 6—*Effect of synthetic xanthines on the dissolution rate of 9,10-dihydroergotoxine in 0.1 N HCl (500 ml.) at 37°, stirring speed 25 r.p.m. Key: ●, 50 mg. dihydroergotoxine; □, 50 mg. dihydroergotoxine and 5.0 g. 7-(2,3-dihydroxypropyl)-theophylline; ◪, 50 mg. dihydroergotoxine and 5.0 g. 7-β-hydroxypropyltheophylline.*

ception of theobromine where the deviation from the 9,10-dihydroergotoxine standard is negligible. It is obvious that caffeine and theophylline have a large influence on the dissolution rate of the ergot alkaloid. Figure 6 shows the same phenomena of increase in dissolution rate on addition of the two theophylline derivatives to the alkaloid, with the extremely water-soluble 7-β-hydroxypropyltheophylline showing the largest rate increase as expected.

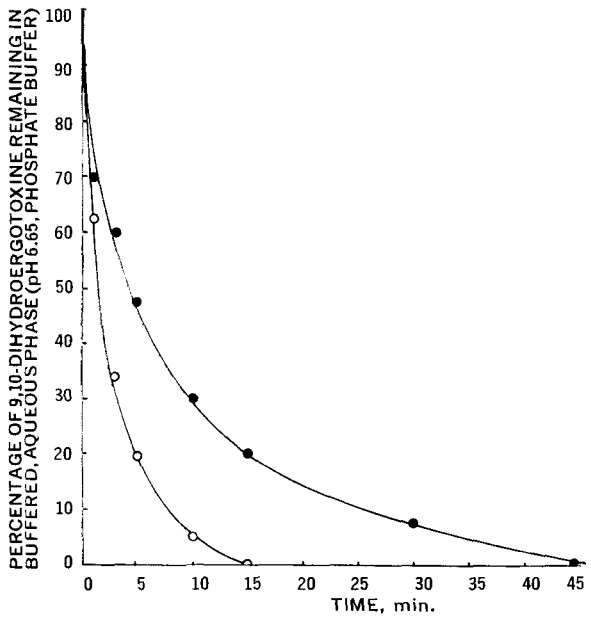

Figure 7—*Plot of amount of 9,10-dihydroergotoxine remaining on partitioning of 10 ml. pH 6.65 buffer with 10 ml. chloroform in a water bath at 6 r.p.m. and 30°. Key: ●, 0.15 mg./ml. dihydroergotoxine; ○, 0.15 mg./ml. dihydroergotoxine plus 15 mg./ml. caffeine.*

Figures 4–6 were derived from a typical experimental run with the only variation being a stirrer speed of 60 r.p.m. in Fig. 4 compared to 25 r.p.m. for the latter two.

Overemphasis should rarely be placed on dissolution rate data alone, but it is plain in this instance that there are significant changes in the dissolution rates on addition of xanthines, excepting theobromine. This information complements the solubility data.

During the course of investigation of the equilibrium distribution between an aqueous phase and chloroform of 9,10-dihydroergotoxine methanesulfonate with and without caffeine, it was noted that the time required for attaining this equilibrium was 15 min. in the former instance and 45 min. in the latter (Fig. 7). These data suggested perhaps the way to relate *in vitro* results to drug absorption was to study the rate of migration from one phase to another rather than look at the equilibrium distribution. The rate at which a drug distributes itself between phases does not necessarily parallel its equilibrium distribution coefficient.

A representative partitioning experiment involving the alkaloid and caffeine is illustrated in Fig. 8. The log of the 9,10-dihydroxyergotoxine methanesulfonate percentage remaining in the aqueous layer (phosphate buffer, pH 6.65) is plotted *versus* time. The graph shows that the rate for the first-order transfer of drug from aqueous phase to chloroform is substantially larger in the presence of caffeine than in its absence. The rate of caffeine partitioning from aqueous to organic phase is apparently the same with and without alkaloid. A 100:1 ratio of caffeine to 9,10-dihydroergotoxine was utilized.

In all instances of partitioning rates determined at pH 1.25 (0.1 *N* HCl), a retardation of transfer of 9,10-dihydroergotoxine from aqueous to organic phase was noted upon inclusion of xanthine (Fig. 9). This phenomenon is unexplained but may be due to some combination of protonation of both species coupled with interaction in acidic media. As drug absorption at this pH is rather unlikely, it was not examined further.

Partitioning rates were run in duplicate—ergotoxine *versus* ergotoxine plus xanthine—and each repeated several times. The experimental conditions approach a first-order process (see *Experimental* section) and give good results for this system. The results paralleled those found with caffeine–ergotamine with the exception of the combination 7-(2,3-dihydroxypropyl)-theophylline:dihydroergotoxine at pH 6.65. This combination exhibited no difference in partitioning rate when compared to dihydroergotoxine. This particular xanthine is insoluble in chloroform which in some way

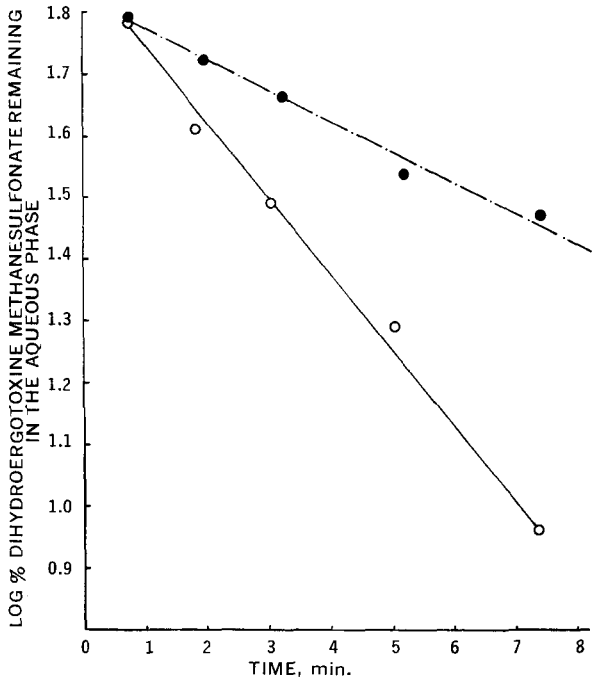

Figure 8—*Effect of caffeine on the partitioning rate of dihydroergotoxine methanesulfonate from an aqueous (phosphate buffer, pH 6.65) to an organic phase (chloroform). Key: ●, dihydroergotoxine, 0.1 mg./ml.; ○, dihydroergotoxine, 0.1 mg./ml.; and caffeine, 10.0 mg./ml.*

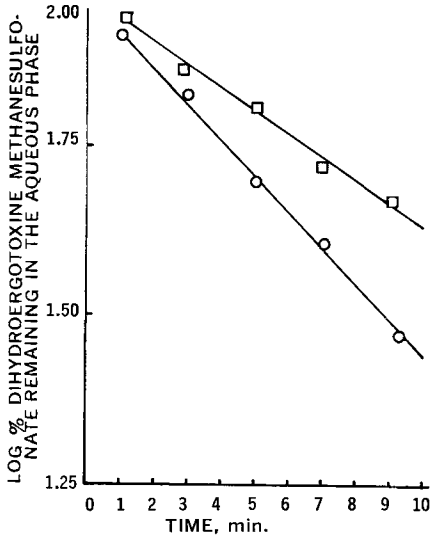

Figure 9—*Effect of 7-(2,3-dihydroxypropyl)-theophylline on the partitioning rate of dihydroergotoxine methanesulfonate from an aqueous phase (0.1 N HCl) to an organic phase (chloroform). Key: ○, dihydroergotoxine methanesulfonate, 0.1 mg./ml.; □, dihydroergotoxine methanesulfonate, 0.1 mg./ml.; and 7-(2,3-dihydroxypropyl)-theophylline, 10.0 mg./ml.*

relates to its inability to affect partitioning at this pH. Solubility problems precluded use of theobromine at this almost neutral pH. The 7-β-hydroxypropyltheophylline was tested in humans with dihydroergocristine, and the same relative results were obtained and are listed in the *Biological* section.

It has been reported by the authors (5) that when a complexing agent is present in partitioning studies, the alkaloid is transferred directly to the organic phase, while in cases of the alkaloid alone a precipitation step precedes this transfer. The increased partitioning at enteral pH (6.65) was probably not due to transfer of complex between phases, but rather to partial prevention of a precipitation step which occurred when the alkaloid itself was present in the aqueous phase.

CONCLUSIONS

The utilization of simple physicochemical studies as a rapid ranking or screening device of known or suspected biologically active agents preceding the tedious and expensive animal and human work is an ultimate goal of these investigations.

In general, the *in vitro* partitioning rate data obtained thus far indicate absorption potentiation at neutral pH should be observed with ergot alkaloids having the polypeptide moiety intact upon addition of certain xanthines such as caffeine. This phenomenon has been examined both for 9,10-dihydroergotoxine methanesulfonate and ergotamine tartrate (5). The rate effect, however, is reversed at gastric pH. Dissolution rate constants are usually increased in 0.1 *N* HCl solutions upon inclusion of xanthine while solubility of the alkaloid at enteral and gastric pH is elevated by most xanthines discussed.

An exceptional degree of success has been achieved in correlation of *in vitro* data with the increased clinical effectiveness of ergot alkaloids in the presence of complexing agents (6, 7). Recent clinical and animal evidence has substantiated the previously obtained *in vitro* results stated in this communication (9).

This ergot alkaloid:xanthine interaction is somewhat unusual as it is an exceptional example where complexation in the gastrointestinal tract leads to enhanced rather than decreased absorption of a medicinal agent.

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