Effect of Complex Formation on Drug Absorption IV

Role of Intragastric Diffusion in the Absorption of Free and Caffeine-Complexed Salicylic Acid from the Rat Stomach

By RICHARD H. REUNING* and GERHARD LEVY†

The effect of complex formation on salicylic acid absorption from the rat stomach has been determined under conditions where the drug solution in the stomach was either stirred by means of a microstirrer or left unstirred. Complexation of part of the salicylic acid with caffeine resulted in a significant decrease in the over-all salicylic acid absorption rate under both conditions. Stirring had no significant effect on the absorption rate of salicylic acid, nor did it affect the absorption rate of salicylic acid in the presence of caffeine. This indicates that diffusion of salicylic acid and/or of the salicylic acid–caffeine complex to the absorbing membranes is not the rate-limiting step in the gastric absorption of salicylic acid under the experimental conditions. It appears, therefore, that the decreased absorption rate of salicylic acid in the presence of caffeine is due to the decreased thermodynamic activity of salicylic acid resulting from complex formation, with the complex being either unabsorbed or more slowly absorbed than free salicylic acid.

IN A RECENT publication in this series (1) it has been reported that the absorption rate of salicylic acid from the *in situ* ligated rat stomach is decreased significantly in the presence of the complexing agent caffeine. It was suggested that this decrease may be due to the following mechanism(s): (*a*) a difference in the partition coefficients of salicylic acid and the salicylic acid–caffeine complex between the aqueous gastric fluids and the lipoid phase of the mucosal barrier, causing the salicylic acid–caffeine complex to be either unabsorbed or more slowly absorbed than free salicylic acid, and/or (*b*) a decreased diffusion rate of the complex through the gastric fluids to the gastric mucosa. The first mechanism was suggested by the relative values of the apparent *in vitro* partition coefficients of the different species (salicylic acid > salicylic acid–caffeine complex > caffeine), while the second mechanism derives support from the quantitative agreement between the results of the absorption experiments and the calculated effect of caffeine on the over-all[1] diffusion rate of salicylic acid in the system (1). The possibility that diffusion to the absorbing membranes is rate-limiting in the absorption of rapidly absorbed weak acids from the ligated rat stomach was suggested originally by Schanker *et al.* (2).

Two experimental approaches were utilized in an attempt to distinguish between these two different mechanisms which could account for the effect of caffeine on salicylic acid absorption. One of these approaches was to reverse the conditions of the original experiment and to determine the effect of salicylic acid on caffeine absorption. If the first mechanism (*i.e.*, modification of the apparent *in vivo* partition coefficient) were operative, one would expect salicylic acid to enhance the over-all absorption rate of caffeine since the apparent partition coefficient of the caffeine–salicylic acid complex is appreciably higher than that of caffeine alone. This approach was tried, but had to be abandoned due to the limited solubility of salicylic acid (which precluded extensive complexation of caffeine) and due to its damaging effect on the gastric mucosa when nearly saturated solutions of the drug were used. The second experimental approach, which is the subject of this report, was to stir the gastric contents by means of a specially designed microstirrer so that diffusion to the absorbing membranes would definitely not be rate-limiting in the absorption process. Thus, if diffusion to the gastric mucosal membrane is a rate-limiting step in the absorption of salicylic acid under unstirred conditions, one would expect the absorption rate of free salicylic acid (*i.e.*, in the absence of caffeine) to increase when the gastric content is stirred. One would also expect that the magnitude of the effect of caffeine on salicylic acid absorption would be changed when the gastric content is stirred because a step other than diffusion to the membranes will then become absorption rate-limiting. On the other hand, if diffusion to the absorbing membrane is not rate-limiting, one would expect that stirring would

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* Fellow of the American Foundation for Pharmaceutical Education.

† To whom requests for reprints should be directed.

[1] In the context used in this paper, "over-all" refers to the combined effect or behavior of free and complexed drug.

have no effect on the absorption rate of salicylic acid in the absence of caffeine, and that the decrease in salicylic acid absorption rate caused by complexation with caffeine will be of equal magnitude under stirred and unstirred conditions.

EXPERIMENTAL

Absorption Rate Measurements—Female Wistar rats weighing 210 to 295 Gm. were used. Food was withheld for 18–24 hr. prior to the experiment. The rats were anesthetized with urethan (1.25 Gm./Kg. i.p.) and remained under anesthesia throughout the absorption experiment. Three different experimental procedures were used; these will be referred to as "unstirred," "static stirrer," and "rotating stirrer." In each experiment 6 ml. of one of the following solutions was introduced into the stomach: (*a*) ethanol 2%, (*b*) salicylic acid 0.1%, ethanol 2.0%, or (*c*) salicylic acid 0.1%, ethanol 2.0%, caffeine 2.75%. All concentrations refer to per cent weight in volume. One-tenth *N* HCl was used as the solvent for each solution.

In the experiments utilizing the "unstirred" procedure a midline incision was made in the abdominal region of the rat and the stomach was exposed. The esophagus was then ligated tightly immediately adjacent to the cardiac sphincter, care being taken not to occlude major blood vessels. A transverse incision was made in the intestine extending about half-way across the lumen diameter and located approximately 0.8 cm. from the pyloric sphincter.[2] The stomach was rinsed once with about 5 ml. of 0.9% sodium chloride solution (37°) by inserting a 16-gauge ball-tipped needle attached to a 5-ml. syringe into the stomach through the incision in the intestine. Six milliliters of the drug solution, initially at 37°, was then injected into the stomach *via* the intestinal incision by means of a 10-ml. syringe fitted with a 23-gauge silver lacrimal cannula.[3] A ligature was placed around the pyloric sphincter and needle prior to the injection in order to prevent leakage. This ligature was tightened further as the cannula was withdrawn. After completion of the above procedure the midline incision was closed with wound clips. One hour after injection the entire stomach was excised and homogenized.[4] The homogenate was then assayed for unabsorbed salicylic acid and ethanol.

The methodology used in the "static stirrer" experiments was similar to that described above, except for the following modifications. A ligature was not initially placed around the esophagus. After rinsing the stomach as previously described, a specially designed microstirrer,[5] shown in Fig. 1, was inserted through the intestinal incision into the stomach. The microstirrer was then secured by tying a ligature around the small intestine at the pyloric sphincter, since this was located directly over

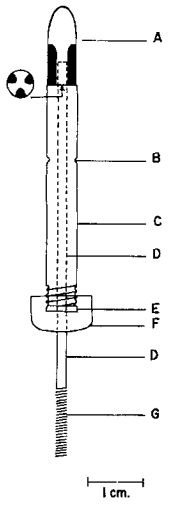

Fig. 1—Cylindrical microstirrer, longitudinal section. Key: A, *revolving Teflon tip with three indentations (shaded portion) which result in a paddlewheel design;* B, *groove for attachment at pyloric sphincter;* C, *Teflon sleeve;* D, *stainless steel shaft;* E, *neoprene O-ring;* F, *plastic screw cap;* G, *flexible shaft. Insert: cross-section of the Teflon tip.*

a groove in the stirrer shaft (Fig. 1, B). The drug solution was then introduced by the technique previously described, except that the lacrimal cannula was inserted into the stomach through a small incision in the esophagus about 0.5 cm. cephalad to the cardiac sphincter. The midline incision through the skin and body wall of the rat was then closed with suture except for an opening through which the stirrer protruded, as shown in Fig. 2. In these "static-stirrer" experiments the microstirrer was not rotated during the absorption period. At the end of this period the stomach with the remaining drug solution and the microstirrer was excised, the stirrer was detached, the stomach and its contents were homogenized, and the homogenate was assayed for unabsorbed salicylic acid and ethanol.

The "rotating stirrer" experiments were performed in the same manner as the "static stirrer" experiments except that after completion of the surgical procedure, the externally located Teflon sleeve of the microstirrer was secured by a clamp, the flexible shaft was attached to a precision laboratory motor,[6] and the stirrer was revolved at 290 ± 10 r.p.m. Stirring was initiated 5 to 7 min. after injection of the solution into the stomach and was continued over the remainder of the 1-hr. absorption period. Observation (through the somewhat transparent gastric wall) of the movement of the small air bubbles within the stomach indicated that adequate mixing of the gastric contents was achieved.

Analytical Methods—Salicylic acid in the gastric homogenate was assayed as described previously (1). Assays were done in duplicate using two 20-ml. portions of the 50 ml. of homogenate. The average recovery of known quantities of drug added to homogenized stomach tissue was 95%. Ethanol was determined by the method of Hoult and Pawan (3). Two aliquots of each homogenate were diluted and assayed, with duplicate determinations being carried out on each diluted aliquot. Standard curves were prepared simultaneously with each set of assays. The average recovery of ethanol from homogenized stomach tissue was 98%.

Statistical Analysis[7]—The salicylic acid and

[2] This incision was needed only for the insertion of the microstirrer in the other two procedures. The same methodology was used when the microstirrer was not inserted in order to obtain a proper control.

[3] This is a soft, blunt-tipped needle which was used to prevent gastric damage.

[4] Multi-mix Homogenizer, Lourdes Instrument Corp., Brooklyn, N. Y.

[5] The authors are indebted to Mr. William Tanski, Jr., Health Sciences Fabrications and Maintenance Laboratory, UNYB, for construction of the microstirrer.

[6] Gerald K. Heller Co., Las Vegas, Nev.

[7] The authors acknowledge the helpful consultation with Dr. Roger L. Priore concerning the statistical analysis.

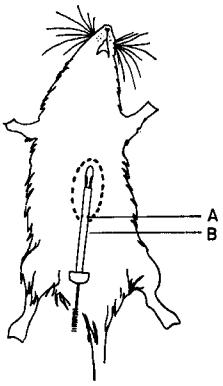

Fig. 2—Approximate position of the stirrer in relation to the rat after completion of surgical and injection procedures. The stippled line indicates the position of the stomach. Key: A, pyloric sphincter tied over groove in Teflon sleeve; B, stirrer sleeve protruding through midline incision.

ethanol absorption data (Table I) were analyzed separately using a 3 × 2 analysis of variance (three experimental conditions, absence and presence of caffeine) with subsequent *F*-tests (4, 5) on the six categories in which both ethanol and salicylic acid were present in the drug solution. The Student *t* test was used to compare the category in which ethanol alone was present with the category in which ethanol and salicylic acid were employed (unstirred condition).

Exclusion of Data—Experiments with the microstirrer occasionally caused pronounced gastric damage. The results of these experiments were not included in the tabulated data. The basis for the exclusion of data was the magnitude of ethanol absorption observed in the "ethanol alone-unstirred" category. All experiments in which ethanol absorption was less than the lowest individual value observed in this category (26%) were excluded.

RESULTS

The results of this study are summarized in Table I. The over-all absorption rate of salicylic acid was decreased by caffeine under all experimental conditions (unstirred, static stirrer, and rotating stirrer). The effect of caffeine was highly significant ($p < 0.01$) in each instance. The absorption rate of ethanol, which was included in the drug solutions as a noncomplexed marker, was not significantly affected by the presence of caffeine under any of the three experimental conditions ($p > 0.05$). Stirring itself had no significant effect on the absorption rate of free salicylic acid, salicylic acid complexed with caffeine, or ethanol (all static stirrer *versus* rotating stirrer experiments, $p > 0.1$). Thus, the decrease in the over-all absorption rate of salicylic acid caused by caffeine was of similar magnitude under both static stirrer and rotating stirrer conditions. Salicylic acid apparently increased the absorption rate of ethanol slightly. This effect was barely significant ($p \sim 0.05$ by *t* test of ethanol-unstirred *versus* salicylic acid, ethanol-unstirred data). It could also be concluded that the presence of the stirrer (whether rotating or not) in the stomach resulted in a significant decrease in the absorption rate of both ethanol and salicylic acid ($p < 0.01$).

Experiments with the microstirrer occasionally caused gastric damage (opaque appearance of the mucosa and/or bleeding). This resulted in a pronounced decrease in the absorption rates of ethanol and salicylic acid. Based on the criterion described under *Experimental*, the following data were excluded: salicylic acid–ethanol, static stirrer, 1; salicylic acid–ethanol–caffeine, static stirrer, 1; salicylic acid–ethanol, rotating stirrer, 7; salicylic acid–ethanol–caffeine, rotating stirrer, 2. It is apparent that the highest incidence of gastric mucosal damage occurred with salicylic acid–ethanol under rotating stirrer conditions. This is probably due to the well known mucosal damaging effect of salicylic acid which may have been enhanced by the mechanical effect of stirring. Some of the instances of mucosal damage may have been due to accidental contact of the rotating part of the stirrer with the gastric mucosa. The average absorption rate of ethanol in the discarded experiments was 19.5 (4.6)%[8] while that in the control experiments was 34.6 (6.7)%.[8] The effect of caffeine on salicylic acid absorption was evident even in the experiments which were discarded because of gastric damage; the difference between the absorption rates of salicylic acid in the absence and presence of caffeine was statistically significant (*t* test, $p < 0.01$) even when none of the collected data were excluded from the analysis.

TABLE I—EFFECT OF STIRRING ON THE ABSORPTION RATE OF SALICYLIC ACID WITH OR WITHOUT CAFFEINE

Initial Compn. of Soln.,[a] %		Condition	% Absorbed[b] in 1 hr. Salicylic Acid	Ethanol
Salicylic acid	0.100	Unstirred	58.1 (6.7)[c]	43.6 (9.3)[c]
Ethanol	2.00			
Salicylic acid	0.100	Unstirred	38.5 (4.9)	42.6 (8.4)
Ethanol	2.00			
Caffeine	2.75			
Salicylic acid	0.100	Static stirrer	46.5 (5.3)	34.4 (5.8)
Ethanol	2.00			
Salicylic acid	0.100	Static stirrer	36.9 (6.6)	41.3 (4.6)
Ethanol	2.00			
Caffeine	2.75			
Salicylic acid	0.100	Rotating stirrer	45.6 (6.1)	34.5 (5.9)
Ethanol	2.00			
Salicylic acid	0.100	Rotating stirrer	32.6 (3.2)	38.1 (6.1)
Ethanol	2.00			
Caffeine	2.75			
Ethanol	2.00	Unstirred		34.6 (6.7)

[a] 0.1 *N* HCl served as the solvent. [b] Mean of 8 animals per group. [c] Standard deviation in parentheses.

DISCUSSION

The decrease in the gastric absorption rate of salicylic acid caused by caffeine was of similar magnitude to that observed in a previous study from this laboratory (1).[9] Also, as in the previous study, caffeine had no significant effect on the absorption rate of ethanol. Ethanol, which is not complexed by caffeine, was included in all drug solutions as a marker to detect any possible changes in the gastric

[8] Standard deviation in parentheses.

[9] It should be noted that the volume of solution introduced into the rat stomach in this study was 6 ml., while the volume used in the previous study was 5 ml. The body weight of the rats used in the previous study was 100–150 Gm. Larger animals had to be used in the present study in order to accommodate the microstirrer.

mucosa and/or its blood supply which might affect the absorption rate of the drugs. Since caffeine did not affect the absorption rate of ethanol but reduced the over-all absorption rate of salicylic acid, it may be concluded that the observed effect is due to an interaction of caffeine with salicylic acid and not to a direct effect of caffeine on the permeability of the gastric mucosa. The complex formation between salicylic acid and caffeine is well known (6) and it has been estimated that about 84% of the salicylic acid in the salicylic acid–caffeine solutions used in this study is complexed with caffeine (1).

Stirring had no significant effect on the absorption rate of ethanol nor on the absorption rate of salicylic acid, with or without caffeine. It appears, therefore, that diffusion of drug through gastric fluids to the membrane is not the rate-limiting step in the absorption of a rapidly absorbed drug such as salicylic acid or a somewhat more slowly absorbed drug, such as ethanol. This conclusion is in agreement with the theoretical considerations outlined by Laidler (7) and Danielli (8), who assumed that when a molecule is diffusing through a nonporous membrane separating two aqueous phases, diffusion through the aqueous phases ordinarily is sufficiently rapid relative to diffusion through the membrane that the former can usually be neglected in the kinetic analysis. Our results indicate that fluid mixing due to body movement of the breathing, although anesthetized, animal is sufficient so that gastric absorption, even without artificial stirring, will not be rate limited ordinarily by intragastric diffusion. Since the magnitude of the caffeine effect on the absorption rate of salicylic acid was similar under "static stirrer" and "rotating stirrer" conditions, it may also be concluded that the absorption of salicylic acid existing in complexed form is not rate limited by the rate of diffusion of the salicylic acid–caffeine complex through the gastric fluids to the absorbing membranes. It should be mentioned, however, that movement of drug to the gastric membranes can be the absorption rate-limiting step if the viscosity of the drug solution is increased considerably (9).

These results are of further interest since they may shed some light on an important aspect of the gastrointestinal absorption of drugs by passive diffusion. Schanker *et al.* (2) have observed an "upper limit" in the relative absorption rate (*i.e.*, per cent per hour) of rapidly absorbed drugs such as salicylic acid, benzoic acid, and *p*-hydroxypropiophenone from the rat stomach. These investigators have suggested that this "upper limit" may be the result of drug diffusion through gastric fluids to the biologic membrane (rather than diffusion through the membrane itself) becoming the rate-limiting step in the gastric absorption of sufficiently rapidly absorbed drugs. They were able to show that gastric blood flow was not a limiting factor in the absorption process. The results of the present investigation, which involved experimental conditions similar to those of the study by Schanker *et al.* (2), show that the rate of movement of drug molecules through the gastric fluids is not rate limiting and cannot account for the upper limit in the gastric absorption of drugs as observed by Schanker and co-workers. A review of the available evidence leads to the conclusion that the rate of transfer of drug from aqueous gastric fluids into the lipoid barrier (real or hypothetical) of the gastric membranes will no longer be absorption rate limiting when the *in vivo* partition coefficient of the drug exceeds a certain value. The quantitative similarity in the maximum relative absorption rate (or, more exactly, the absorption rate constant) of diverse passively absorbed drugs suggests that the rate-limiting step is one of diffusion; the results of the present study indicate that the locus of the rate-limiting diffusion is somewhere within the gastric membrane barrier or possibly in the mucous layer immediately adjacent to the gastric epithelium (particularly in the gastric pits), rather than the gastric fluids. From a practical point of view, it appears that increasing the lipoid–water partition coefficient of drugs by molecular modification may be of limited value in increasing absorption rate when the drug itself already has a relatively high partition coefficient. It must be realized, of course, that when a drug is given in solid form rather than in solution, it is dissolution, rather than membrane permeation, which will usually be rate limiting in the absorption process.

The reason for the effect of salicylic acid on ethanol absorption is not readily apparent. This relatively small effect, which was only of borderline statistical significance, was not observed in a previous study (1) and also was not apparent in the "static stirrer" and "rotating stirrer" experiments of the present study. It is likely to have been due to chance, particularly since ethanol absorption appears to have been more variable than salicylic acid absorption.

The decrease in the absorption of salicylic acid and ethanol in the presence of the stirrer may have been due to the manipulation involved while inserting the stirrer into the stomach. It does not appear to be due to the extra distention of the stomach by the added volume of the stirrer ($\sim$0.75 cm.^{3}) and a resulting decrease in blood flow through capillaries in the gastric mucosa, since Schanker *et al.* (2) found that aniline clearance from blood to stomach was essentially constant over a range of gastric volumes encompassing the volume used in the present study.

Certain theoretical calculations outlined previously (1) suggest that the salicylic acid–caffeine complex may be absorbed as such and at a rate intermediate between that of free salicylic acid and free caffeine. These calculations had to be based on assumptions which, unfortunately, are rather speculative. The possible presence of other than 1:1 complexes of salicylic acid and caffeine, the possible existence of caffeine dimers and larger aggregates, the possibility of competitive binding of one or both components of the complex by membrane constituents, and the likelihood of partial dissociation of the complex at the surface of the biologic membranes (10) make it impossible to reach a definitive conclusion with respect to the absorption of the salicylic acid–caffeine complex as such. A relatively conclusive experiment, namely the determination of the effect of complexation with salicylic acid on the absorption rate of caffeine, could not be carried out because of the technical difficulties outlined in the introductory discussion. Studies are now in progress with systems which, it is hoped, do not involve these difficulties.

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Effect of Polymorphism on the Absorption of Chloramphenicol from Chloramphenicol Palmitate

By ARMANDO J. AGUIAR, JOHN KRC, JR., ARLYN W. KINKEL, and JOSEPH C. SAMYN

The effect of polymorphism on the availability of chloramphenicol from chloramphenicol palmitate was studied. *In vivo* absorption studies in humans, following single oral doses of the ester, indicate that the polymorphic state of chloramphenicol palmitate influences significantly the blood levels obtained. The use of enzymatic hydrolysis as an *in vitro* method for predicting blood levels is evaluated. The utility of X-ray diffractometry, melting point determinations, and infrared spectroscopy in qualitative and quantitative analysis of the polymorphic forms is discussed. In addition to the two previously reported polymorphs of this drug designated as polymorphs A and B, a third polymorph, form C, is described.

PHYSICAL PROPERTIES such as particle size, solubility, rates of solution, aggregation of primary particles, and wettability have been shown by various workers (1–7) to influence the absorption and therapeutic efficacy of relatively insoluble drugs. The polymorphic state of a drug can also be an important factor (8) since differences in the free energy of polymorphs influence some of these properties. Higuchi (9) has suggested that these differences may appreciably affect their physiological activity.

The present study is concerned with an evaluation of the effect of polymorphic state on the absorption of chloramphenicol from chloramphenicol palmitate. The study was also designed to determine the usefulness and limitations of commonly applied analytical techniques such as X-ray diffractometry, infrared spectroscopy, and melting point techniques in identifying and characterizing the polymorphs of chloramphenicol palmitate. Furthermore, the utility of enzymatic hydrolysis as an *in vitro* criterion for predicting *in vivo* blood levels is examined.

This report deals with the properties and absorption characteristics of polymorphs A and B of chloramphenicol palmitate. Data on the third polymorph (form C) will be presented in a subsequent publication.

Chloramphenicol palmitate was synthesized by Edgerton (10) as a tasteless derivative of chloramphenicol. Glazko *et al.* (11) showed that the intact esters are poorly absorbed from the intestinal tract and must first be hydrolyzed by the esterases in the small intestine before any significant absorption can take place. The rate of hydrolysis of the ester is governed by the rate of solution which, in turn, is dependent on factors such as primary particle size, state of aggregation of primary particles, but most important, as this study shows, on the polymorphic form.

PAST WORK ON THE POLYMORPHISM OF CHLORAMPHENICOL ESTERS

Milosovich (12), studying the physical stability of chloramphenicol palmitate suspensions, suggested the existence of two polymorphs of this drug. Subsequently, Tamura and Kuwano (13) and Maruyama *et al.* (14) reported their work on the two polymorphs and an additional amorphous glassy phase.

Almirante and his co-workers (15) showed that chloramphenicol stearate also exists in two forms, one of which gave good blood levels regardless of particle size. These authors also showed that the presence or absence of a wetting agent had no appreciable effect on the blood levels obtained.

Recently Menachemoff (16) reported that the hydrolysis time of chloramphenicol palmitate is the

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