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The absorption from the gut of quinine and chlorpheniramine given with various anionic agents

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The absorption of quinine and chlorpheniramine with anionic agents was examined in the rat rectum, small intestine and stomach. The enhancement of absorption was related to partition behaviour, to the organic solvent, and surface activity of ion-pair complexes, but it seems that with results from these properties alone it is not possible to explain the apparent increase in the *in situ* absorption of amines. In the presence of anionic agents, the absorption of amines was enhanced at all sites examined and kinetically the uptake to the mucosal membrane was increased in the rectum and small intestine. The effect of anionic agents on the uptake of amines was greater in the rectum than in the small intestine and this may be related to differences in the nature of the gut wall.

We have previously reported that drug absorption behaviour from the rat rectum is more consistent with the pH-partition hypothesis than is drug absorption from the small intestine (Kakemi, Arita & Muranishi, 1965).

According to this hypothesis, it would be expected that pharmaceutically important amines and quaternary ammonium compounds having pK_a values greater than 8 would be poorly absorbed at physiological pH range. However when such ionized drugs are associated with counter-ions, they become lipophilic and are readily transported across the intestinal membrane (Kakemi, Sezaki & others, 1969).

There is evidence that such an enhancement of absorption of cationic drugs could better be related to their surface activity or to the binding behaviour to the rectal mucosal membrane than to their apparent partition coefficients (Kakemi & others, 1969; Fiese & Perrin, 1969).

We have now looked further into the mechanism of ion-pair transport across the gastrointestinal barrier paying special attention to the site specificity.

MATERIALS AND METHODS

Materials. Quinine hydrochloride (pK_a 8·4), chlorpheniramine maleate (pK_a 9·2), saccharin sodium, trichloroacetic acid (TCA) and sodium lauryl sulphate (SLS) were of commercial quality. All other materials used were of reagent grade.

Determination of physicochemical properties

Apparent partition coefficients. Drugs were initially dissolved in the same isotonic buffer solutions as used in the absorption experiments. Five ml of the buffered solution was added to an equal volume of organic solvent previously saturated with the same buffer solution and equilibrated at 37°. The aqueous phases were analysed

for amines before and after equilibration and the apparent partition coefficients calculated by difference. Benzene and chloroform were used as organic solvents.

Surface tension. Surface tension was measured at 25° ± 0·5° with a du Nöuy interfacial tensiometer.

Analytical methods

All spectrophotometric analyses were made with a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer.

Quinine. A few ml of sample was placed in a glass-stoppered test tube and that half volume of 5N sodium hydroxide and 7 ml of ethylene dichloride were added. After the tube had been shaken for 15 min and centrifuged, the aqueous phase was removed. Five ml of the organic phase was transferred to a glass-stoppered test tube containing 4 ml of N hydrochloric acid. After the tube had been shaken for 15 min and centrifuged, the aqueous phase was separated and measured at 250 nm.

Chlorpheniramine. One half ml of sample was placed in a glass-stoppered test tube and 0·5 ml of N sodium hydroxide and 7 ml of cyclohexane were added. After the tube had been shaken for 15 min and centrifuged, the aqueous phase was removed. Five ml of organic phase was transferred to a glass-stoppered test tube containing 5 ml of 0·1N hydrochloric acid. After the tube had been shaken for 20 min and centrifuged, 3 ml of aqueous phase was separated. This solution was diluted with 5 ml of 0·1N hydrochloric acid and measured at 265 nm.

Sulphaguanidine was diazotized, coupled with 2-diethylaminoethyl-1-naphthylamine, and the coloured material extracted with isoamyl alcohol after the addition of sodium chloride. The organic phase was assayed spectrophotometrically at 550 nm.

Procedure of absorption experiments

Stomach. The *in situ* ligation technique of Schanker, Shore & others (1957) was employed. Sulphaguanidine was used as volume change indicator.

Intestine. The techniques using the perfused small intestine (Koizumi, Arita & Kakemi, 1964) and rectum (Kakemi & others, 1965) were employed. The perfusion rate was 10 ml/min and the volume of perfusate was 40 ml for the small intestine and 30 ml for the rectum.

Procedure for measuring the amount of drug passing from the gut wall into perfusion fluid

The procedure was the same as the *in situ* method for the intestinal absorption study. Quinine, 4 mg in 0·5 ml of 30% *NN'*-dimethylacetamide solution, was administered intravenously to the rat via the femoral vein, and the small intestine was perfused first with pH 7·0 isotonic phosphate buffer and then with 0·4mM SLS solution at about 1 ml/min. The perfusion solution was collected every 9 min, analysed for quinine and the apparent rate quinine entered the perfusate was calculated.

RESULTS

Effect of anionic agents on the partition of quinine

Apparent partition coefficients of quinine at pH 5·5 and 7·0 are summarized in Table 1. It is apparent that all the anions substantially increased the partition

Table 1. *Effect of anionic agents on the apparent partition coefficient of quinine at* 37°.

Drug	pH	Organic solvent	Apparent partition coefficient
Quinine	7	C_6H_6	2·28
Quinine + SLS	7	C_6H_6	355
Quinine	7	$CHCl_3$	37·1
Quinine + saccharin	7	$CHCl_3$	79·0
Quinine + TCA	7	$CHCl_3$	56·7
Quinine	5·5	C_6H_6	0·19
Quinine + SLS	5·5	C_6H_6	114
Quinine	5·5	$CHCl_3$	1·29
Quinine + saccharin	5·5	$CHCl_3$	23·9
Quinine + TCA	5·5	$CHCl_3$	33·6

Concentration of drugs: Quinine (0·2mM), SLS (0·4mM), Saccharin (5mM), TCA (10mM).

coefficients of quinine even at a pH as low as 5·5. With the quinine-saccharin system, saccharin extraction was increased in proportion to the increment of quinine. This does not coincide with our previous findings in the *in situ* absorption experiment in which quinine, but not saccharin, absorption was increased by an ion-pair formation (Kakemi & others, 1969).

Surface tension

Fiese & Perrin (1969) have shown that the absorption of dextromethorphan was accelerated in the presence of anions and the absorption rate was parallel to the surface activity of the species rather than their lipid solubility. Surface tensions measured in isotonic phosphate buffer solutions are shown in Table 2. Combinations

Table 2. *Surface tensions of isotonic phosphate buffers containing various drugs at* 25° ± 0·5°.

Drug	Surface tension mNm^{-1} (dynes/cm)	Drug	Surface tension mNm^{-1} (dynes/cm)
Water	73·8		
pH 7·0 Buffer		pH 5·5 Buffer	
Quinine (0·2mM)	73·2	Chlorpheniramine (1mM)	73·5
Quinine (0·2mM) + SLS (0·4mM)	33·9	Chlorpheniramine (1mM) + TCA (75mM)	56·5
Quinine (0·2mM) + saccharin (5mM)	73·1	TCA (75mM)	73·6
Quinine (0·2mM) + TCA (10mM)	73·3		
SLS (0·4mM)	34·7		

Drug concentration is shown in parentheses.

of quinine-SLS and chlorpheniramine-TCA increased the surface activities suggesting interfacial ion-pair formation (Patel & Zografi, 1966). Though the critical micellar concentration of SLS has been reported to be 8·1mM in pure water, this value is decreased in the isotonic buffer solution to around 0·4mM. The solution containing 0·2mM quinine and 0·4mM SLS is cloudy at room temperature (20°) but becomes clear at 37°. Quinine thus seems to form micelles and insoluble complexes in the range of experimental conditions used.

Effect of anionic agents on the rectal absorption of quinine

SLS and saccharin greatly increased the absorption of quinine, but the remarkable effect of TCA in the increasing partition was not reflected on the luminal transfer (Table 3). At pH 5·5, the control value for the quinine absorption was so small that no increase in absorption was observed except with SLS.

Table 3. *Effect of anionic agents on the rectal absorption of quinine at pH* 7·0.

Drug	% Absorbed/h	*P* value
Quinine	7·7 ± 1·6	—
Quinine + SLS	11·7 ± 3·2	0·001
Quinine + saccharin	10·7 ± 2·3	0·01
Quinine + TCA	7·4 ± 1·8	N.S.*
Quinine after pretreatment with SLS	8·0 ± 0·9	N.S.*

* Not significant.
Values are expressed as mean ± s.d. of mean.
Drug concentration is the same as shown in Table 1.

Doluisio, Crouthamel & others (1970) examined the effect of membrane storage on the kinetics of drug absorption. They concluded that, in a three compartment model of drug absorption, when accumulation in the membrane phase was appreciable, bi-exponential disappearance occurred whereas when membrane accumulation was low or absent, monoexponential disappearance occurred. Since back diffusion of quinine to gut lumen from blood was negligible in the rat rectum, their method of analysis was applied to investigate which of the following transfer steps was influenced by the anionic agents. In the scheme, c_a, c_b and c_c represent the drug concentration in the luminal phase, membrane phase and blood respectively.

$$c_a \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} c_b \xrightarrow{k_{23}} c_c$$

The results of the analyses are summarized in Fig. 1 and Table 4. It is evident that anionic agents greatly increase k_{12}, the rate constant from the luminal phase to the absorptive membrane. In other words, the affinity of quinine for the membrane was greatly increased by the presence of anions.

Effect of SLS on the absorption of quinine from the rat small intestine

To investigate site specificity of the ion-pair complex, the absorption of quinine

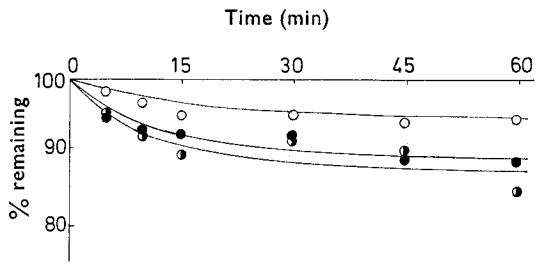

FIG. 1. Logarithmic plot of quinine absorption from rat rectum at pH 7·0. Quinine ○, Quinine + SLS ●, Quinine + Saccharin ◑.

Table 4. *Kinetic data for the disappearance of quinine from rat rectum.*

Drug	$k_{12} \times 10^2$	$k_{23} \times 10^3$	$k_{21} \times 10$
Quinine	0·46	6·16	0·84
Quinine + SLS	1·71	8·99	1·94
Quinine + saccharin	2·20	7·99	2·22

Rate constants (min^{-1}) are calculated by the method of Doluisio & others (1970).
Drug concentration is the same as shown in Table 1.

with SLS, the combination giving the most striking absorption enhancement effect, was chosen and the small intestinal absorption was examined. Surprisingly, the effect of SLS is relatively small in this part of the intestine in comparison with the rectum (Table 5), although SLS seems to have a tendency to decrease the initial stage of absorption.

Table 5. *Effect of SLS on the absorption of quinine from rat small intestine at pH* 7·0.

Drug	Perfusion period (min)	% Absorbed
Quinine	15	23·7 ± 2·8
Quinine + SLS	15	29·0 ± 4·0
Quinine	60	45·7 ± 2·9
Quinine + SLS	60	50·5 ± 3·0

Values are expressed as mean ± s.d. of mean.
Drug concentration is the same as shown in Table 1.

Effect of SLS on the rate of passage of intravenously administered quinine into the rat small intestine

Sodium taurocholate increases the rate of passage of intravenously administered sulphaguanidine into the rat small intestine and this effect is caused by the direct action of endogenous surface-active agent on the absorptive membrane (Kakemi, Sezaki & others, 1970). To ascertain whether the enhancement effect of SLS on the quinine absorption was due to a direct action of SLS on the membrane permeability, the rate that intravenously given quinine passed into the rat small intestine was measured. The results (Fig. 2) rule out the possibility of such an action. The rate

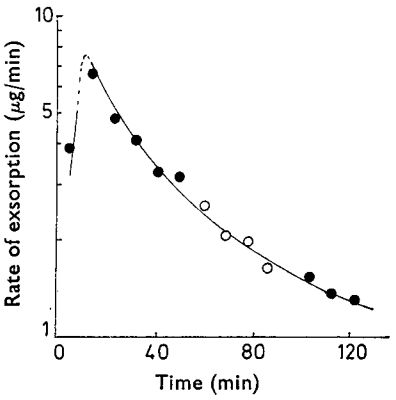

FIG. 2. Effect of SLS on the rate of passage of intravenously administered quinine to rat small intestine. Control ●, With 0·4mM SLS ○.

was hardly affected by the intestinal perfusion of SLS solution of concentration equal to that which enhanced the absorption of quinine from the gut.

This finding was further confirmed by the experiment in which 0·4mM SLS solution was perfused through the rectum for a certain time and completely removed by gentle washing with the isotonic buffer solution before the regular absorption experiment. Absorption of quinine from the pretreated rectum did not significantly differ from the control value (Table 3).

Effect of trichloroacetate on the absorption of chlorpheniramine from the rat stomach

The apparent partition coefficients and the absorption rate from the stomach are illustrated in Table 6. Contrary to rectal absorption (Table 3), absorption was increased in proportion to the apparent partition coefficient and surface tension in this part of the alimentary tract. It seems probable that the trichloroacetate effect in the enhancement of absorption of amine drugs would largely be produced in the stomach.

Table 6. *Effect of TCA on the apparent partition coefficients and the gastric absorption of chlorpheniramine at pH* 4·0, 5·5 *and* 7·0.

	Partition coefficient		Rate of absorption (%/h)	
pH	without TCA	with TCA	without TCA	with TCA
4·0	0·11	2·67	0·9 ± 0·7	1·3 ± 1·1
5·5	2·71	6·47	3·2 ± 1·1	6·2 ± 0·8
7·0	18·9	18·6	19·4 ± 3·0	20·3 ± 3·0

Buffer components: pH 4·0 (citric acid–HCl–NaOH–NaCl), pH 5·5 and pH 7·0 (NaH_2PO_4–Na_2HPO_4).
Drug concentration: Chlorpheniramine (1mM), TCA (75mM).
Organic solvent in the partition experiments: mixture of chloroform and cyclohexane (1 : 1 by volume).

DISCUSSION

Several investigators have contributed much to the knowledge of the mechanism of ion-pair absorption. In studies on the absorption of dextromethorphan (Fiese & Perrin, 1969) and tetracycline (Perrin & Vallner, 1970) from the rat stomach, absorption appeared to be better related to the surface activity of the components.

Previously Kakemi & others (1969) demonstrated that certain organic anions, such as lauryl sulphate and saccharinate could enhance the gastrointestinal absorption of otherwise poorly absorbable pharmaceutical amines and that the enhancement in the absorption was not by the anionic but by the cationic component of an ion-pair complex. It was therefore proposed following *in vitro* experiments that the increase of the drug binding to the absorptive membrane was responsible for the absorption enhancement by the ion-pair formation in perfusion medium.

However, there seems to be some controversy about the mechanism of absorption of such ion-pair complexes of drugs that are absorbed apparently by passive diffusion.

The data presented herein support the view that although the increase in lipophilicity and the decrease in surface tension of the medium due to ion-pair complex formation are in most instances requisite to the enhancement of absorption, these changes in physicochemical properties alone fail to explain the apparent increase in

the *in situ* absorption data of amines as a result of ion-pair complex formation. For instance, the saccharinate anion, which does not lower surface tension could increase the rectal absorption of quinine to the same extent as lauryl sulphate.

Compartmental kinetic analyses of biexponential lumen phase data in the *in situ* rectal absorption of quinine by the method of Doluisio & others (1970) revealed that k_{12}, the rate constant from the gut lumen to the absorptive membrane, was increased by four to five-fold in the presence of the anions investigated. This phenomenon can be interpreted as an increase of the binding tendency of the drug to the absorptive membrane. Binding or "accessibility" seems to be favoured by the ion-pair formation and the more pronounced biexponential characteristics in the disappearance of quinine from the rectal lumen was noticed.

This view was further substantiated by the results of the experiments in which the amount of intravenously administered drug being taken up by perfusion of the gut was measured. The apparent rate that the drug entered into the intestinal lumen was hardly affected by the intestinal perfusion of SLS solution, thus ruling out the possibility of a general increase in permeability caused by SLS, an anionic component of the ion-pair.

Such an absorption enhancement effect was absorptive-site dependent. Generally, absorption enhancement by the ion-pair formation was obvious in the stomach and in the rectum, whereas the effect was not remarkable in the small intestine. It is probable that the enhancement of absorption may occur by the increase of drug concentration in the absorptive membrane surface which is related not only to the physicochemical properties of the ion-pair complexes in the medium but to the chemical nature of the surface of the absorptive site.

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