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Enhanced migration of the ionized forms of acidic drugs from water into chloroform in the presence of phospholipids

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The transfer process of various acidic drugs from water into chloroform containing phospholipids has been examined using a non-emulsifying system. The ionized forms of the drugs which have little lipid solubility show a marked increase in transfer to the chloroform phase containing lecithin at the physiological pH of the small intestine. The pH-profiles of salicylic acid, *p*-aminobenzoic acid and sulphisoxazole transfer are in good agreement with available *in situ* absorption data. These results suggest a role for phospholipids in the intestinal absorption of ionized acidic drugs which has not been clearly apparent with pH-partition hypothesis alone.

Kakemi, Arita & others (1969) demonstrated that some qualitative discrepancies from the pH-partition hypothesis developed by Brodie, Schanker & others (1958) were interpretable by a binding process to the mucosal surface. Although the absorption of the ionized forms of drugs was also interpreted by this binding factor, there is a need to clarify the kind of component of the mucous membrane that can interact with drugs to facilitate the absorption from the alimentary tract.

Solute transfer at the oil–water interface has been used to elucidate the mechanism of drug absorption (Perrin, 1967; Doluisio & Swintosky, 1964). LeFevre, Jung & Chaney (1968) showed that the apparent chloroform–water partition coefficient of glucose was markedly raised by addition to the chloroform of any of the major fractions of the phospholipids extracted from human erythrocyte stroma. Sears (1970) also showed that lecithin or cephalin could facilitate the water and salt transport across a pentanol–water interface. We have investigated the absorption mechanism of the ionized form of acidic drugs by examining drug partitioning between water and chloroform containing lecithin.

MATERIALS AND METHODS

All the drugs except metoclopramide were of analytical grade and were obtained commercially. Metoclopramide was the gift from Fujisawa Pharmaceutical Industry Ltd., Osaka. Egg phosphatidylcholine (Merck Ltd.), phosphatidylethanolamine and phosphatidylserine (Tokyo Kasei Co.) were used without further purification. [^{14}C]Salicylic acid was purchased from Japan Radio Isotope Association.

All buffer ingredients and organic solvent were of reagent grade.

Determination of drug partitioning

Chloroform saturated with buffer and buffer saturated with chloroform were used to minimize volume changes due to mutual miscibility. Phospholipid was dissolved into chloroform at 10 mg/ml. Citric acid–dibasic sodium phosphate systems were

used for buffer solutions of pH 2 to 5, dibasic sodium phosphate–sodium biphosphate systems for pH 6 to 8 and a sodium carbonate–sodium bicarbonate system for pH 9 and 10.

The ionic strength of the aqueous medium was adjusted to 0·04 by the dilution of the buffer. Equal volumes of the two solvents with phospholipid and drug were placed in a glass-stoppered test-tube and rocked for 100 min at 20 cycle/min. An apparatus modified from that of Doluisio & Swintosky (1964) was used. Rocking the two phase system led to slight emulsification, this broke on overnight standing. The temperature was always 25°. The difference in amount of drug present in the aqueous phase before and after equilibrium was taken as the amount partitioning into the organic phase containing phospholipids. Radioactivity assay of the lipid phase confirmed the validity of this procedure.

Estimation

The distribution of the labelled salicylic acid was estimated by counting the [^{14}C] content in a Beckman liquid scintillation counter by the dual-channel ratio method of Hendler (1964), using Bray's (1960) scintillation mixture.

Because of the quenching by chloroform, samples were taken to dryness before addition of the scintillation fluid. Non-labelled salicylic acid and other drugs were determined spectrophotometrically (Brodie, Schanker & others, 1957) with a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer.

RESULTS AND DISCUSSION

The effect of lecithin (phosphatidylcholine) on the transfer of various drugs from the pH 6·5 aqueous phase to the chloroform phase is shown in Table 1. Acidic drugs in column 1 which are negatively charged at this pH showed marked increases in their transfer to chloroform containing lecithin.

This phenomenon suggests an interaction of phospholipids and acidic drugs might occur on absorption of the drugs through the intestinal tract. While the transfer of *m*-nitroaniline, which is undissociated and has a high lipid solubility, is not influenced by addition of lecithin, unionized moieties of sulphanilamide and sulphaguanidine show slightly enhanced migration to lecithin-containing chloroform. Basic drugs such as metoclopramide and procaine amide hydrochloride show slightly decreased transfer to lecithin-containing chloroform.

Table 1. *Effect of lecithin on the transfer of various drugs from water into chloroform.* Drug concentration 0·1 mM except isonicotinic acid 0·03 mM. Drug dissolved in Sørensen phosphate buffer M/60 pH 6·5 at 25°.

Drug	Transfer (%) Lecithin	Control	Drug	Transfer (%) Lecithin	Control
Salicylic acid	76·1	0	*m*-Nitroaniline	96·6	97·5
Sulphisoxazole	75·7	6·7	Sulphanilamide	5·0	1·6
Isonicotinic acid ..	54·4	0	Sulphaguanidine ..	5·5	0
p-Aminobenzoic acid ..	53·2	1·6			
p-Acetaminobenzoic acid	54·8	0	Metoclopramide ..	55·7	59·7
p-Aminohippuric acid ..	45·6	0	Procaine amide	0	3·1

These results suggest an ionic interaction between negatively charged drugs and lecithin is involved in the transfer process. It is generally known that lecithin can interact with anionic substances and form a relatively stable complex in an organic solvent (Hirt & Berchtold, 1959; Horton & McClure, 1971).

Table 2. *Effect of various phospholipids on the transfer of* [^{14}C]*salicylic acid from water into chloroform.* Drug concentration 0·1 mM. Salicylic acid dissolved in Sørensen phosphate buffer M/60 pH 7·0 at 25°.

Phospholipids	Radioactivity ratio Chloroform/water	Foam/water
None	0·0004	—
Phosphatidylcholine (PC)	1·35	2·14
Phosphatidylethanolamine (PE) ..	0·01	—
Phosphatidylserine	0·01	—
PC : PE=3 : 2	0·10	—
PC : PE=2 : 3	0·02	—

The effect of various phospholipids on the transfer of [^{14}C]salicylic acid is shown in Table 2. Phosphatidylcholine in chloroform enhanced the migration of salicylic acid from water into the organic phase several thousand times more than occurred when the control with no phospholipids was used. The control result (0·0004) is quite satisfactory, assuming that only the unionized form of salicylic acid can migrate to chloroform phase. When phosphatidylethanolamine or phosphatidylserine is added to chloroform phase, the apparent partition coefficient of salicylic acid becomes about 25 times greater than the control value. The mixture of phosphatidylcholine and phosphatidylethanolamine had little effect on the enhancement of salicylic acid transfer. This phenomenon is probably due to the reduced effective concentration of lecithin's positively charged head groups exposed at the aqueous interface. The highest radioactivity was found in the foam phase created at an oil–water interface. This result also supports the above explanation.

In Fig. 1 is shown the rate of approach to equilibrium. Sulphisoxazole and *p*-amino-

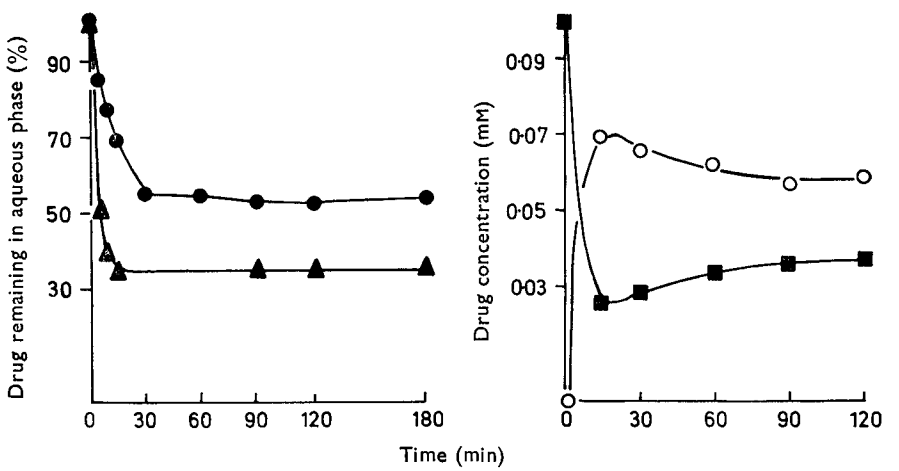

FIG. 1. Rate of interphase transport of drugs by egg lecithin (10 mg/ml). ●—● Sulphisoxazole in aqueous phase. ▲—▲ *p*-Aminobenzoic acid in aqueous phase. ■—■ Salicylic acid in aqueous phase. ○—○ Salicylic acid in chloroform phase.

benzoic acid transfer take 30 and 15 min respectively to reach equilibrium. The difference in time to reach equilibrium is reflected by the extent of the amount transferred to lecithin-containing chloroform.

The shorter the equilibration time, the larger the extent of transfer. The results indicate a strong interaction of the phospholipids and the ionized form of acidic drugs rather than a simple physical distribution. The time course of [^{14}C]salicylic acid transfer versus rocking time shows maximum extent of transfer at 15 min; after that, the extent of transfer is gradually reduced. An overshoot and a release from a chloroform–lecithin–salicylic acid system may be caused by a change of character of the emulsion entrapping the salicylic acid. The same curve was obtained theoretically using a hexadecane–water interface and an adsorbed gelatin film (Ghanem, Higuchi & Simonelli, 1969).

The dependence of drug transfer on the concentration of lecithin in the chloroform phase was determined over the range of 0·1–50·0 mg/ml (Fig. 2A). Saturation of the drug transfer process could be demonstrated by increasing the amount of lecithin at a constant drug concentration. The 10 mg/ml concentration was chosen since maximal rates of transfer were achieved at this concentration and higher concentrations provided excessive emulsion problems.

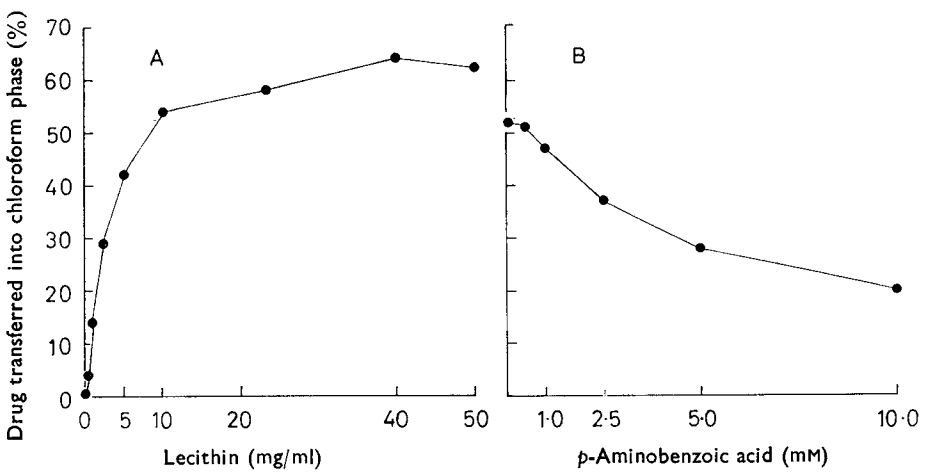

FIG. 2 A. *p*-Aminobenzoic acid migration into chloroform, as a function of lecithin concentration. Sørensen phosphate buffer M/60 pH 7·0.

B. Equilibrium distribution of *p*-aminobenzoic acid in chloroform–water two-phase system, as a function of *p*-aminobenzoic acid concn (lecithin 10 mg/ml). Sørensen phosphate buffer M/60 pH 7·0.

At a constant concentration of lecithin, the percentage transfer of drug decreases as its concentration increases (Fig. 2B). Although constant percentage transfer of drug was obtained below a 0·1 mM drug concentration, we could not find a strict stoichiometric relation between the interaction of lecithin and drug. These results suggest the solubilization of anionic substances by the phospholipid micelles in the bulk organic phase that would be expected to have the choline groups centrally orientated.

Fig. 3 shows pH-profiles of the percentage transfer into the chloroform phase of salicylic acid, *p*-aminobenzoic acid and sulphisoxazole which agree with those of available published and unpublished *in situ* absorption data from our laboratory

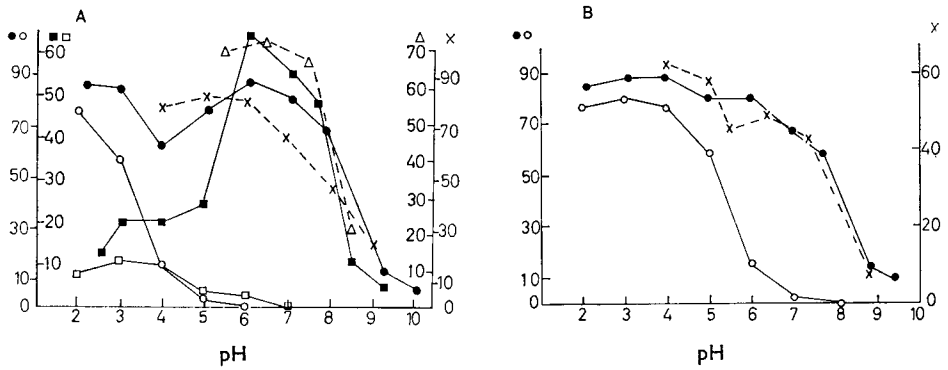

FIG. 3. Comparison of *in situ* absorption data and *in vitro* transfer experiment with 10 mg/ml lecithin in chloroform from solutions of various pH for (A) salicylic acid (○●×) and *p*-amino-benzoic acid (□■△) and (B) sulphisoxazole. ● and ■ Transport with 10 mg/ml lecithin in chloroform. ○ and □ Transport without lecithin. ×, △ % absorbed through the rat intestinal tract in 1 h. Left hand ordinates: drug transferred in chloroform phase (%). Right hand ordinates: drug absorbed through intestinal tract (%).

(Kakemi & others, 1969). At about pH 6 both the *in vitro* transfer process and the *in situ* absorption process have the maximum rate. At this pH, the amount of the drugs unionized is negligible. The possibility exists that some of the stages in the intestinal absorption process will be limited by the extent of interactions of the ionized forms of acidic drugs and membrane component phospholipids. Since the ionized forms have little or no lipid solubility, we have been unable to predict the intestinal absorption of acidic drugs from the ordinary pH-partition hypothesis. By using lecithin-containing chloroform as an organic phase, a reasonable correlation of absorption data and percentage transfer of acidic drugs has been obtained.

REFERENCES

BRAY, G. A. (1960). *Analyt. Biochem.*, **1**, 279–285.

BRODIE, B. B., SCHANKER, L. S., SHORE, P. A. & HOGBEN, C. A. M. (1957). *J. Pharmac. exp. Ther.*, **120**, 528–539.

BRODIE, B. B., SCHANKER, L. S., TOCCO, D. J. & HOGBEN, C. A. M. (1958). *Ibid.*, **123**, 81–88.

DOLUISIO, J. T. & SWINTOSKY, J. V. (1964). *J. pharm. Sci.*, **53**, 597–601.

GHANEM, A. H., HIGUCHI, W. I. & SIMONELLI, A. P. (1969). *Ibid.*, **58**, 165–174.

HENDLER, R. W. (1964). *Analyt. Biochem.*, **7**, 110–120.

HIRT, R. & BERCHTOLD, R. (1959). *Experientia*, **15**, 373–375.

HORTON, A. W. & MCCLURE, D. W. (1971). *Biochim. biophys. Acta*, **225**, 248–253.

KAKEMI, K., ARITA, T., HORI, R., KONISHI, R., NISHIMURA, K., MATSUI, H. & NISHIMURA, T. (1969). *Chem. Pharm. Bull., (Tokyo)*, **17**, 255–261.

LEFEVRE, P. G., JUNG, C. Y. & CHANEY, J. E. (1968). *Archs Biochem. Biophys.*, **126**, 664–676.

PERRIN, J. (1967). *J. Pharm. Pharmac.*, **19**, 25–31.

SEARS, D. F. (1970). *Agents and Actions*, **1**, 183–189.