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Research Papers

CONTRIBUTION TO THE INTESTINAL ABSORPTION OF ERGOT PEPTIDE ALKALOIDS

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SUMMARY

The rate of disappearance of radiolabelled ergot peptide alkaloids from the lumen o the gastrointestinal tract of rats, in situ, was studied. The intestinal absorption of thes compounds is slow and incomplete. Disappearance rate is increased considerably by addi tion of mucolytic enzymes and by high local drug concentrations. A two compartmen model of absorption kinetics of the ergot peptide alkaloids, depending on two parame ters, is described.

Based on these findings it is suggested that the mucus layer is the rate-limiting barrie for the intestinal absorption of ergot peptide alkaloids due to interactions via hydroge bonds.

INTRODUCTION

The intestinal absorption of hydrogenated ergot peptide alkaloids varies generall between about 10 and 30% (Eckert et al., 1978). Prior to this study the exact reason fo the incomplete absorption of these compounds was not known.

Earlier unpubiished studies showed that neither the solubility nor the dissolution rat of the ergot peptide alkaloids in aqueous buffer solution are the rate-limiting factors o the multiple step process of intestinal absorption. In order to gain more insight into th absorption process and to find out the rate-limiting parameters involved in the kinetics o disappearance of some labelled ergot peptide alkaloids, studies were carried out using th in situ perfusion of rat intestine. Previous publications have dealt with the use of an i situ model for studying the dynamics of the absorption process (Schanker et al., 195 1960; Koizumi et al., 1964a and b; Doluisio et al., 1969; Tsuji et al., 1978a and b). Th perfusion technique has the dual advantages of both pH and drug concentration contr in the perfusion liquic..

Until now the lipoidal epithelial membrane of the microvilli has been considered as the principal barrier to drug absorption. The absorption kinetics of most drugs are in good agreement with the predictions of the pH-partition hypothesis of Schanker et al. (1958). Exceptions can be accounted for by the existence of additional more hydrophilic barriers composed of mucoproteins and mucopolysaccharides with a gel-like structure (Kakemi et al., 1969; Ho et al., 1977; Tsuji et al., 1978b; Nimmerfall et al., 1980).

The so-called unstirred layer together with the goblet cell mucin and the glycocalix or fuzzy coat form the primary diffusion barrier for the absorption of compounds present in the intestinal fluid (Ito, 1965; Forstner et al., 1973; Westergaard et al., 1974; Wilson et al., 1974; Clamp et al., 1978; Edwards, 1978). The transport through these layers may determine to a large extent the absorption rate of large basic molecules (Levine et al., 1961; Trier, 1969; Ho et al., 1977; Higuchi, 1979). The mucus barrier should become more permeable to drug molecules after partial degradation of the mucus structure by mucolytic enzymes (Nakamura et al., 1976). To prove the validity of this hypothesis for ergot compounds, the effect of papaine and hyaluronidase added to the perfusion liquid was studied. Furthermore, the influence of the local drug concentration on the disappearance rate was examined.

MATERIALS AND METHODS

Materials

The labelled compounds were prepared in the Synthetic Tracer Laboratory of Sandoz, Basle. Radiochemical purity of the compounds was checked by TLC (Eckert et al., 1978) and amounted at least to 95%. Papaine purum and cysteine puriss were purchased from Fluka, Buchs.

Animals

Male SPF rats weighing 250–300 g were used for the perfusion experiments. Food was withdrawn 20 h prior to the experiments, but water was allowed ad libitum.

In situ recirculation procedure

The recirculation method was based on that of Schanker (1958, 1960) and Tsuji (1978a). Anesthesia was introduced with ether and maintained with pentobarbital, 12 mg/kg, i.p., approximately 15 min prior to surgery. The small intestine was exposed by a midline abdominal incision, and two silicone tubes were inserted through small slits at the duodenal and ileal ends. The tubes were secured by ligations with silk suture and the intestine was returned to the abdominal cavity. The pylorus and the colon were closed off by ligatures. In most of the experiments the bile duct was not ligated. The small intestine was rinsed carefully with about 100 ml of saline solution at 37°C. The silicone tubes were then connected to a masterflex pump and a thermostat respectively. The perfusion solution consisting of the labelled drug dissolved in 0.9% saline was maintained at 37°C and was recirculated at a rate of 13 ml/min. The total volume was kept constant by supply of perfusion liquid from a graduated funnel placed vertically in the perfusion system by means of a T-tube as shown in Fig. 1. Three samples of 0.05 ml each were collected from the perfusion fluid at time intervals of 10 min and analyzed for radioactivity by a liquid scintillation counter.

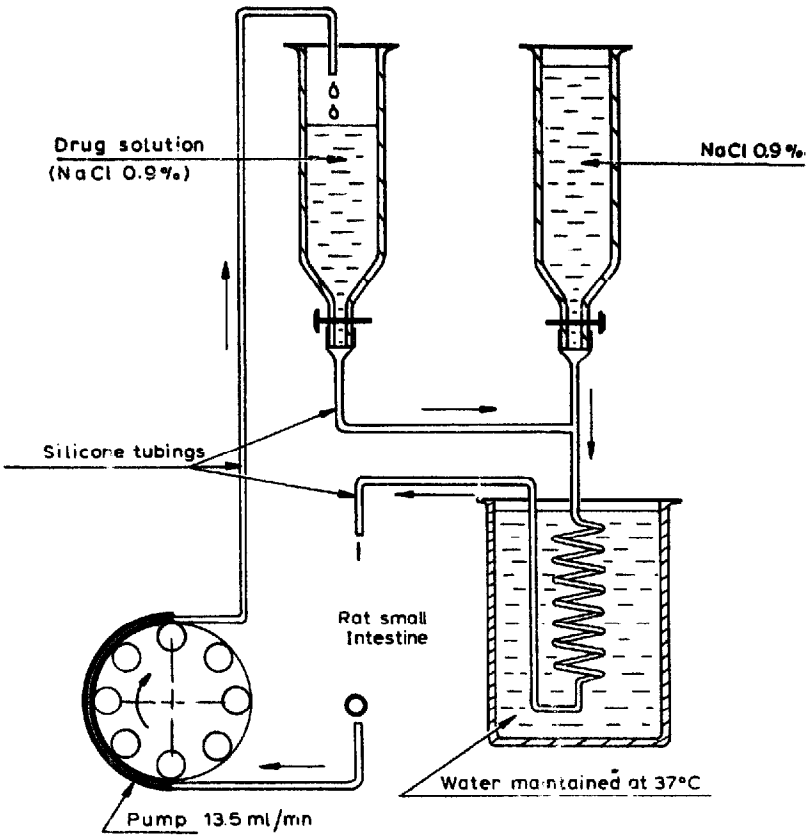

Fig. 1. Experimental scheme for in situ perfusion of rat small intestine.

RESULTS AND DISCUSSION

Fig. 2 shows a semi-logarithmic plot of per cent of the initial concentration of 4 different drugs vs time. Both ergot compounds, ergotamine and dihydroergotamine, reveal a characteristic absorption pattern. The rate of drug disappearance does not follow the pattern of first-order kinetics. A small amount, about 5–10%, disappearing rapidly in the first 10 min whereas the principle amount remains unabsorbed as indicated by the horizontal terminal slope of the curve. This pattern points to an important factor that inhibits the intestinal absorption of ergot peptide alkaloids. Guanfacinum and Ketotifen, unlike the ergot compounds, present a different slope which after extrapolation to infinity yields about 100% absorption.

A compartment model describing the absorption kinetics of ergot peptide alkaloids from the perfusion liquid can be considered. The model takes into account the diffusion across the gut wall of a fraction of the initial amount A_0 of the drug and the simultaneous transformation of another fraction into an unabsorbable form A′ (for instance by absorption to or by interaction with the mucus gel). The amount of unchanged drug A present in the perfusion liquid at a given time t can be expressed by the following equation:

$$-\frac{dA}{dt} = K_a(C - C_b) + K_i A \qquad (1)$$

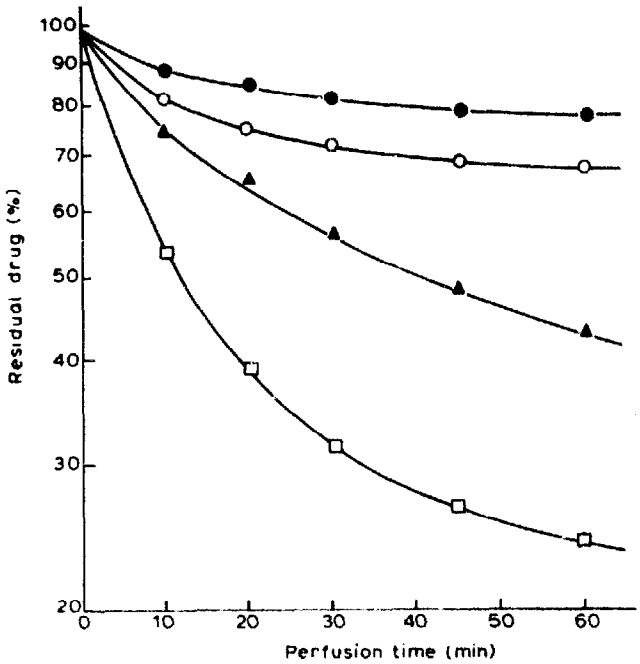

Fig. 2. Disappearance of radioactivity, expressed as per cent of initial concentration of drug, from the lumen of the small intestine of rats perfused in situ. ●, [^{3}H]dihydroergotamine; ○, [^{3}H]ergotamine; ▲, [^{14}C]guanfacinum; □, [^{3}]ketotifen. Initial concentration 1 μg/ml.

where C and C_b represent the drug concentration in the perfusion liquid and in the plasma compartment respectively. K_a is the intrinsic rate constant (ml · min^{-1}). K_i is the first-order transformation rate constant (min^{-1}). C can be expressed by the ratio of the amount A of drug and the volume V of the perfusion liquid as follows:

$$-\frac{dA}{dt} = K_a\left(\frac{A}{V} - C_b\right) + K_i A \tag{2}$$

when V = const and $C >> C_b$ Eqn. 2 becomes

$$-\frac{dA}{dt} = \left(\frac{K_a}{V} + K_i\right) A \tag{3}$$

or

$$\frac{A}{A_0} = e^{-K_{app} \cdot t} \tag{4}$$

where A_0 = amount of drug in solution at t = 0, K_{app} = the apparent first order absorption rate constant which can also be expressed as

$$K_{app} = \frac{K_a}{V} + K_i \tag{5}$$

The formation of the unabsorbable form of the drug in the perfusion compartment can be described as:

$$\frac{dA'}{dt} = K_i \cdot A \tag{6}$$

or, according to Eqn. 4

$$dA' = A_0 K_i \, e^{-K_{app} \cdot t} \, dt \tag{7}$$

The variation of the concentration of total radioactivity in the perfusion compartment depending on A and A′ can be described by the following equation:

$$\frac{C}{C_0} = \frac{A}{A_0} + \frac{A'}{A_0} \tag{8}$$

or using Eqns. 4 and 8

$$\frac{C}{C_0} = \left(1 - \frac{K_i}{K_{app}}\right) e^{-K_{app} \cdot t} + \frac{K_i}{K_{app}} \tag{9}$$

At $t = \infty$ follows

$$\frac{C_\infty}{C_0} = \frac{K_i}{K_{app}} = \frac{1}{1 + K_a/V \cdot K_i} \tag{10}$$

then Eqn. 9 becomes

$$\frac{C}{C_0} = \left(1 - \frac{C_\infty}{C_0}\right) e^{-K_{app} \cdot t} + \frac{C_\infty}{C_0} \tag{11}$$

and

$$K_i = K_i = K_{app} \cdot \frac{C_\infty}{C_0} \tag{12}$$

and

$$K_a = V \cdot K_{app} \left(1 - \frac{C_\infty}{C_0}\right) \tag{13}$$

In other words the time course of the concentration of total radioactivity in the perfusion compartment depends on two parameters, K_{app} and C_∞/C_0.

In Fig. 3 a simulation of the kinetics of drug disappearance according to Eqn. 11 is shown. Fig. 4 demonstrates that a satisfactory fit can be obtained for the experimental results according to Eqn. 11.

The strong influence of a partial degradation of the mucus layer by papaine which has mucolytic and proteolytic activities on the absorption rate of dihydroergonine is pre-

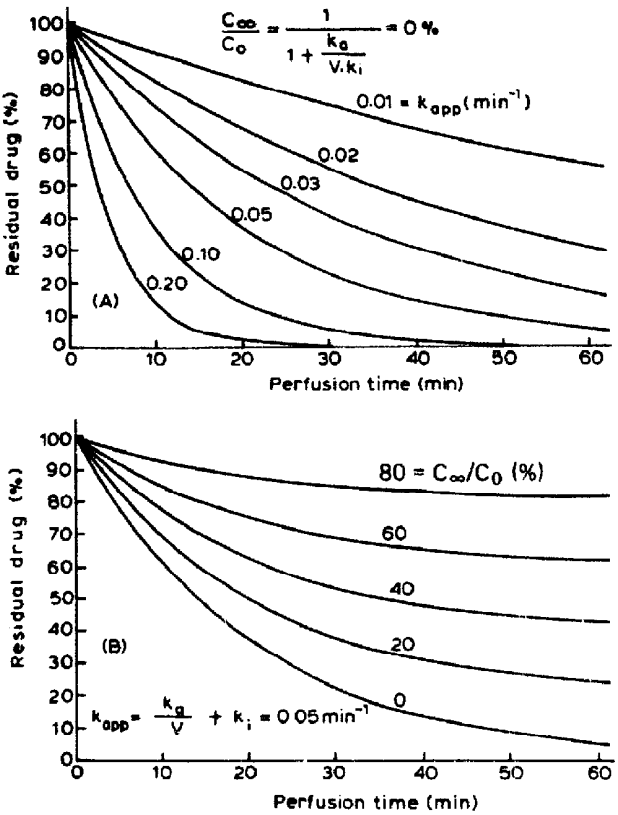

Fig. 3. Simulation of the kinetics of drug disappearance from the lumen of small intestine of rats perfused in situ. A: K_{app} (apparent absorption rate constant) is varying. B: C_∞/C_0 (drug concentration ratio extrapolated to infinity) is varying.

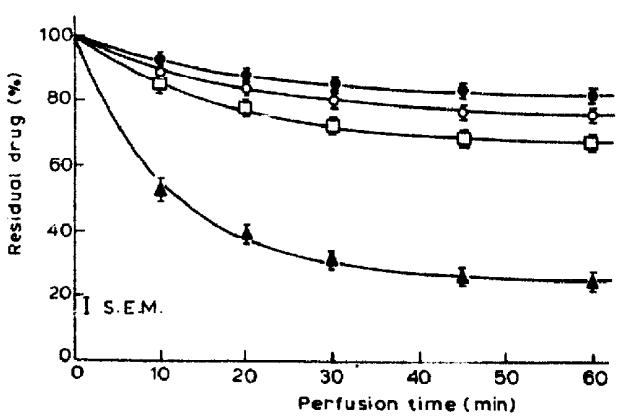

Fig. 4. Curve fitting of disappearance of radioactivity from the lumen of small intestine of rats perfused in situ. •, [^{3}H]dihydroergonine; ○, [^{3}H]DHP 28-377; □, [^{3}H]DZ 26-474; ▲, [^{3}H]ketotifen. Initial concentration 1 μg/ml. The solid lines are computer generated curves based on Eqn. 12.

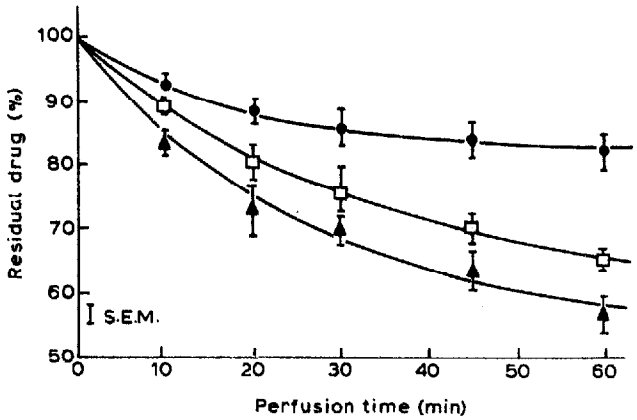

Fig. 5. Influence of a mucolytic enzyme on the kinetics of disappearance of radioactivity from the lumen of small intestine of rats perfused in situ. ●, [^{3}H]dihydroergonine, initial concentration 1 μg/ml; □, [^{3}H]dihydroergonine + papaine, initial concentration 5 mg/ml; ▲, [^{3}H]dihydroergonine + papaine + cysteine, initial concentration 5 mg/ml.

sented in Fig. 5. A similar effect has been observed with hyaluronidase as shown in Table 1. These findings suggest that the mucus layer (unstirred layer plus glycocalix) is indeed the rate-limiting barrier for the absorption of ergot peptide alkaloids.

In earlier investigations in rats with p.o. and i.d. administration of ergot peptide alkaloids it was shown that a higher percentage of the amount applied is absorbed when the dose was increased to up to several milligrams per rat. This prompted us to investigate the influence of a higher local drug concentration on the kinetics of drug disappearance in the perfusion model. Fig. 6 shows the considerable increase of the absorption rate of [^{3}H]-dihydroergonine from different segments of the rat intestine as soon as the concentration

TABLE1

INFLUENCE OF HYALURONIDASE AND PAPAINE ON [^{3}H]DN 16-457 ABSORPTION PARAMETERS DURING IN SITU PERFUSION OF THE SMALL INTESTINE OF RATS

Drugs	Parameters				
	K_a/V (min^{-1})	K_i (min^{-1})	$K_a/V \cdot K_i$	K_{app} (min^{-1})	C_∞/C_0 (%)
DN 16-457 ch (1 μg/ml)	0.0101	0.0475	0.212	0.0576	82.54
DN 16-457 ch + hyaluronidase (1 mg/ml)	0.0165	0.0415	0.396	0.0580	71.63
DN 16-457 ch + papaine (5 mg/ml)	0.0125	0.0178	0.702	0.0302	58.70
DN 16-457 ch + papaine + cysteine (5 mg/ml)	0.0179	0.0205	0.871	0.0384	53.50

The parameters were obtained by resolving Eqn. 11 with an iterative least-squares method.

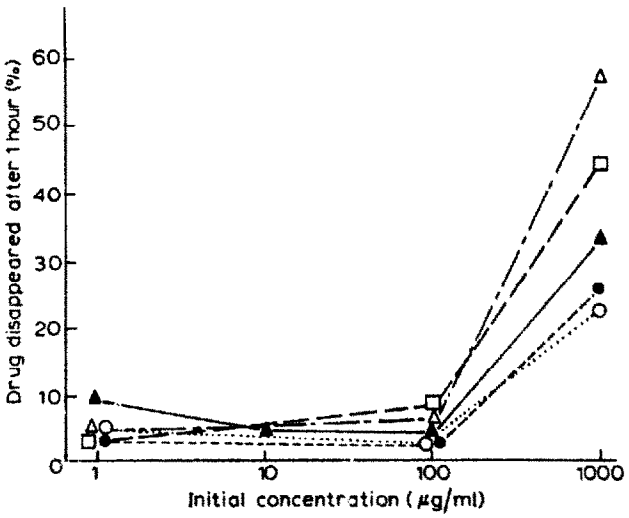

Fig. 6. Concentration dependence of the kinetics of disappearance of ^{3}H-labelled dihydroergonine from different segments of the intestine of rats perfused in situ. ▲———▲, duodenum; ○· · · · ·○, upper jejunum; ●- - - - -●, lower jejunum; △- - - - -△, ileum; □- - - - -□, colon.

reaches 1 mg/ml. Fig. 7 demonstrates that this concentration effect is not restricted to dihydroergonine, but seems to be a common feature of ergot peptide alkaloids.

Metabolic degradation of the ergot compounds in the course of the perfusion experiments could have been a possible explanation of the special absorption pattern. However, analysis of the perfusion liquid performed at the end of the experiments by 4 different methods (TLC, HPLC, molecular sieve chromatography on Sephadex and ultracentrifugation) yielded no degradation products.

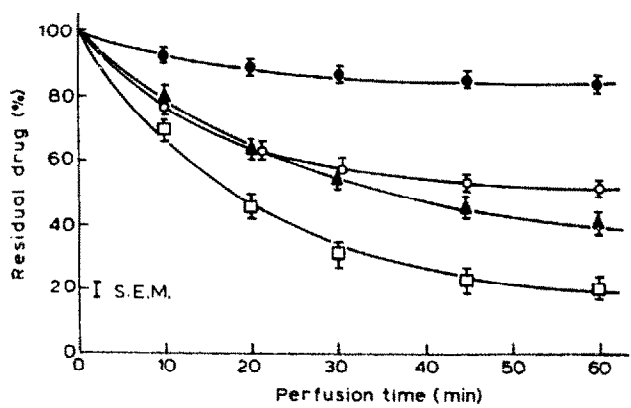

Fig. 7. Influence of high drug concentrations on the kinetics of disappearance of radiolabelled ergot peptide alkaloids from the lumen of the small intestine of rats perfused in situ. ●, [^{3}H]dihydroergonine, initial concentration 1 µg/ml; ▲, [^{3}H]dihydroergonine + dihydroergonine, initial concentration 1 mg/ml; ○, [^{3}H]dihydroergonine + dihydroergocristine, initial concentration 1 mg/ml; □, [^{3}H]-dihydroergonine + DHP 28-377, initial concentration 1 mg/ml.

It is not yet clear if the breaching of the mucus barrier under the influence of a high local drug concentration is due to a saturation phenomenon or to some alteration of the 3-dimensional gel-like structure of the mucus. The basic skeleton of ergot peptide alkaloids, also called ergot peptines, is composed of a lipophilic lysergic acid half and a more hydrophilic cyclic peptide part (Rutschmann et al., 1978). Consequently the total molecule exhibits surface-active properties. Furthermore, it can be derived from the rather complex structure of the ergot peptide alkaloids that these molecules can develop intramolecular and intermolecular hydrogen bonds as well and can also build up hydrogen bonds with surrounding macromolecules such as mucoproteins and mucopolysaccharides which would account for the special absorption pattern of these compounds.

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REFERENCES

Clamp, J.R., Allen, A., Gibbons, R.A. and Roberts, G., Chemical aspects of mucus. Br. Med. Bull., 34 (1978) 25–41.

Doluisio, J.T., Billups, N.F., Dittert, L.W., Sugita, E.T. and Swintosky, J.V., Drug absorption I. An in situ rat gut technique yielding realistic absorption rates. J. Pharm. Sci., 58 (1969) 1196–1200.

Eckert, H., Kiechel, J.R., Rosenthaler, J., Schmidt, R. and Schreier, E., Biopharmaceutical aspects. Analytical methods, pharmacokinetics, metabolism and bioavailability. In Berde, B. and Schild, H.O. (Eds.), Handbook of Experimental Pharmacology, Springer-Verlag, Berlin, 1978, Vol. 49, new series, pp. 719–813.

Edwards, P.A.W., Is mucus a selective barrier to macromolecules? Br. Med. Bull., 34 (1978) 55–56.

Forstner, J., Taichman, N., Kalnins, V. and Forstner, G., Intestinal goblet cell mucus: isolation and identification by immunofluorescence of a goblet cell glycoprotein. J. Cell Sci., 12 (1973) 585–602.

Higuchi, W.I., Rate limiting steps in drug absorption. Abstracts of the Int. Conf. on Drug Absorption, 1979, Edinburgh, p. 14.

Ho, N.F.H., Park, J.Y., Morozowich, W. and Higuchi, W., Physical model approach to the design of drugs with improved intestinal absorption. In Roche, E.B. (Ed.), Design of Biopharmaceutical Properties through Prodrugs and Analogs, Am. Pharm. Ass. Acad. Pharm. Sci. 1977, 136–227.

Ito, S., Structure and function of the glycocalyx. Fed. Proc., 28 (1969) 12–25.

Kakemi, K., Arita, T., Hori, R., Konishi, R., Nishimura, K., Matsui, H. and Nishimura, T., Absorption and excretion of drugs XXXIV. An aspect of the mechanism of drug absorption from the intestinal tract of rats. Chem. Pharm. Bull., 17 (1969) 225–261.

Koizumi, T., Arita, T. and Kakemi, K., Absorption and excretion of drugs. XIX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides. (1). Absorption from the rat stomach. Chem. Pharm. Bull., 12 (1964a) 413–420.

Koizumi, T., Arita, T. and Kakemi, K., Absorption of drugs. XX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides. (2). Absorption from rat intestine. Chem. Pharm. Bull. 12 (1964b) 421–427.

Levine, R.R. and Pelikan, E.W., The influence of experimental procedures and dose on the intestinal absorption of an onium compound, benzomethamine. J. Pharm. Exp. Ther., 131 (1961) 319–327.

Nakamura, J., Yoshizaki, Y., Yasuhara, M., Kimura, T., Muranishi, S. and Sezaki, H., Role of membrane compounds, glycocalyx and lipid in absorption of water-soluble dyes from the rat small intestine. Chem. Pharm. Bull., 24 (1976) 691–697.

Nimmerfall, F. and Rosenthaler, J., Significance of the goblet-cell mucin layer, the outermost luminal barrier to passage through the gut wall. Biochem. Biophys. Res. Commun. 94 (1980) 960–966.

Rutschmann, J. and Stadler, P.A., Ergot alkaloids and related compounds. Chemical background. In Berde, B. and Schild, H.O. (Eds.), Handbook of Experimental Pharmacology, Springer-Verlag, Berlin, Vol. 49, new series, pp. 29–85.

Schanker, L.S., Tocco, J., Brodie, B.B. and Hogben, C.A.M., Absorption of drugs from the rat small intestine. J. Pharm. Exp. Ther., 123 (1958) 81–88.

Schanker, L.S. and Tocco, D.S., Active transport of some pyrimidines across the rat intestinal epithelium. J. Pharm. Exp. Ther., 128 (1960) 115–121.

Trier, J.S., The surface coat of gastrointestinal epithelial cells. Gastroenterologia, 56 (1969) 618–622.

Tsuji, A., Miyamoto, E., Kagami, I., Sakaguchi, H. and Yamana, T., G.I. absorption of beta-lactam antibiotics I. Kinetic assessment of competing absorption and degradation in the G.I. tract. J. Pharm. Sci., 67 (1978a) 1701–1704.

Tsuji, A., Miyamoto, E., Hashimoto, N. and Yamana, T., Absorption of beta-lactam antibiotics II. Deviation from pH-partition hypothesis in penicillin absorption through in situ and in vitro lipoidal barriers. J. Pharm. Sci., 67 (1978b) 1705–1711.

Westergaard, H. and Dietschy, J.M., Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine, J. Clin. Invest., 54 (1974) 718–732.

Wilson, F.A. and Dietschy, J.M., The intestinal unstirred layer: its surface area and effect on active transport kinetics. Biochem. Biophys. Acta 363 (1974) 112–126.