International Journal of Pharmaceutics, 4 (1980) 271–279

Research Papers

MECHANISM FOR THE INDUCEMENT OF THE INTESTINAL ABSORPTION OF POORLY ABSORBED DRUGS BY MIXED MICELLES I. EFFECTS OF VARIOUS LIPID–BILE SALT MIXED MICELLES ON THE INTESTINAL ABSORPTION OF STREPTOMYCIN IN RAT

NORIYUKI MURANUSHI, MARIKO KINUGAWA, YASUKO NAKAJIMA, SHOZO MURANISHI and HITOSHI SEZAKI

Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto (Japan)

(Received July 26th, 1979)
(Accepted October 31st, 1979)

SUMMARY

The effects of various lipid–bile salt mixed micelles on the intestinal absorption of streptomycin were investigated using in situ closed-loop method in the rat. Lipids used were fatty acids, glycerides, oleyl alcohol and methyl oleate. Mixed micelles composed of monoolein or unsaturated fatty acids markedly enhanced the absorption of streptomycin in the large intestine. On the other hand, saturated fatty acids caused a small enhancement of the absorption. Triolein, diolein, oleyl alcohol and methyl oleate had no enhancing effect on the absorption.

To clarify the difference in the enhancing effects of monoolein, unsaturated fatty acids and saturated fatty acids, the interaction of the drug with mixed micelles, the absorbability of lipids and the alteration of the mucosal membrane permeability induced by mixed micelles were investigated. The alteration of the mucosal membrane permeability was examined by an exsorption experiment. The difference in the enhancing effects was not attributed to the interaction or the absorbability of lipids, but a close correlation was found between the enhancing effects and the alteration of the mucosal membrane permeability. Monoolein or unsaturated fatty acids mixed micelles markedly increased the mucosal membrane permeability, while bile salt or saturated fatty acid mixed micelles caused small or no alteration of the permeability. The enhancement of the intestinal absorption by mixed micelles was mostly due to the alteration of the mucosal membrane permeability.

INTRODUCTION

In the previous paper (Muranishi et al., 1979), we studied the effect of monoolein– or oleic acid–bile salt mixed micelles on the intestinal absorption of streptomycin and

gentamicin, which are poorly absorbable drugs. It was found that the absorption of these drugs was greatly enhanced in the presence of monoolein or oleic acid mixed micelles, while bile salt had a small effect on the absorption. The enhancing effect of mixed micelles was clearly elicited in the large intestine and the effect was reversible. The intestinal absorption of heparin, a macromolecular substance, was also promoted by the addition of monoolein mixed micelles (Tokunaga et al., 1978).

In the present investigation, streptomycin was chosen as a poorly absorbable drug and the mechanism for the enhancement of the intestinal absorption induced by mixed micelles was investigated by studying the effects of various lipid mixed micelles on the absorption or on the mucosal membrane permeability.

MATERIALS AND METHODS

Materials. Streptomycin was supplied from Takeda Chemical Industries. Monoolein of high purity grade (Nikko Chemicals) was used. Sodium taurocholate was synthesized according to the method of Norman (Norman, 1955). All other chemicals were of analytical grade and were obtained from Nakarai Chemicals.

Absorption experiment. Male Wistar albino rats weighing 200–250 g were used in all experiments. The preparation of test solution and the procedure of loop method were the same as those reported in the previous paper (Muranishi et al., 1979). Mixed micellar solution was prepared with pH 6.5 isotonic phosphate buffer solution (NaH_2PO_4–Na_2HPO_4). The test solution was introduced into the intestinal loop in a vol. of 4 ml to the small intestine and 2 ml to the large intestine per 200 g body weight. At the end of an absorption study, the concentration of the drugs in plasma or in the withdrawn solution from the intestinal loop was determined.

Interaction of streptomycin with mixed micelles. With equilibrium dialysis cells which had two chambers and were separated by a Visking tube membrane, the interaction of streptomycin with mixed micelles was studied. Equal volumes of mixed micellar solution (40 mM) and the drug solution (2 mg/ml) were separately placed into the chambers and the cells were shaken overnight at 37°C. Binding (%) was calculated using the difference of the drug concentrations on both sides.

Exsorption experiment. To examine the effect of mixed micelles on the mucosal membrane permeability, an exsorption study was performed using the large intestine. The operation was the same as the absorption experiment. Since perfusion was carried out with Perista mini-pump (Mitsumi Scientific). Sulfanilic acid, 34.8 mg in 1 ml of 50% N,N'-dimethylacetamide solution, was administered to the rat via the femoral vein, and immediately the intestinal loop was perfused with isotonic buffer solution at the rate of 2 ml/min. The perfusate was collected every 4 ml with a fraction collector, and the absorption rate was calculated from the amount of sulfanilic acid in the solution. To investigate the effects of various mixed micellar solutions on the permeability of the mucosal membrane, the perfusate was replaced by the other new solution at definite times.

Analytical methods. Streptomycin: streptomycin was determined by a modification of the method of Faure (Faure and Blanquet, 1964). Sample solution (0.5 ml) was alkalinized with 1 ml of 2 N NaOH in a brown tube and 5 ml of sodium β-naphthoquinone-4-sulfonate solution (20 μg/ml) was added. After 1 h the fluorescence of the

reaction product was measured on a Hitachi 512 spectrofluorometer using activation and emission wavelengths of 355 and 430 nm, respectively. Streptomycin in plasma was determined by the paper disc method using *Bacillus subtilis* PCI 219 as the test organism. Sulfanilic acid: sulfanilic acid was diazotized and coupled with 2-diethylamino-ethyl-1-naphthylamine. The colored material was extracted with isoamyl alcohol by the addition of sodium chloride and the organic phase was determined spectrophotometrically at 560 nm. Monoolein: monoolein was extracted with chloroform by the addition of sodium chloride and determined by the method of van Handel (van Handel, 1961) with some modification. The chloroform was removed and the monoolein was hydrolyzed. The product glycerol was oxidized by sodium periodate to formaldehyde. The latter was colored with chromotropic acid and determined at 570 nm after adding thiourea. Fatty acid: fatty acids were extracted with chloroform by the addition of N HCl and determined by the method of Duncombe (Duncombe, 1963).

RESULTS

Effects of various lipid–bile salt mixed micelles on the intestinal absorption of streptomycin

Mixed micellar solutions were prepared by dissolving lipid in sodium taurocholate (NaTC) micellar solution containing the drug and by sonicating the mixture at 37°C for 3 min. Table 1 shows plasma concentrations of streptomycin at 1 h after administration to the small intestine in various formulations. The previous paper indicated that the 1-h plasma concentration was on the increasing portion of plasma level (Muranishi et al., 1979). Plasma concentration was negligible with no adjuvant in the buffer solution and a small increase of plasma concentration was seen in the case of the 40 mM bile salt micellar solution. On the other hand, a significant increase in 1-h sample was elicited after administration of the 40 mM mixed micellar solution which was composed of monoolein,

TABLE 1

PLASMA CONCENTRATION OF STREPTOMYCIN AT 1 h AFTER ADMINISTRATION OF VARIOUS FORMULATIONS INTO THE SMALL INTESTINE

Composition [a]	$C_{m:n}$ [b]	μg/ml ± S.E.M. [c]	
None		<1.5	(5) [d]
40 mM NaTC		3.8 ± 0.2	(5)
40 mM NaTC + 40 mM monoolein		11.6 ± 0.6	(4)
40 mM NaTC + 40 mM oleic acid	$C_{18:1}$	11.9 ± 1.6	(7)
40 mM NaTC + 40 mM lauric acid	$C_{12:0}$	11.6 ± 0.8	(5)
40 mM NaTC + 40 mM palmitic acid	$C_{16:0}$	8.0 ± 1.2	(5)
40 mM NaTC + 40 mM stearic acid	$C_{18:0}$	4.0 ± 0.1	(4)

[a] All formulations were prepared in pH 6.5 isotonic phosphate buffer solution. Administered dose of streptomycin was 8 mg/200 g rat.

[b] m = number of carbon; n = number of double bonds.

[c] Figures represent the mean ± S.E.

[d] Figures in parentheses refer to the number of animals.

oleic acid or lauric acid. The enhancement of the absorption was rather small when lipid was replaced by palmitic acid or stearic acid.

In the previous paper, it was demonstrated that the enhancement of the absorption occurred to an effective degree using even the 10 mM mixed micellar solution in the large intestine. The subsequent absorption experiments in the large intestine were performed using the 10 mM mixed micellar solution. Table 2 shows the 15-min plasma concentration of streptomycin after administration of various mixed micellar solutions into the large intestine. The 15-min concentration was almost regarded as the peak of the plasma level as described in the previous paper. Streptomycin in the 15-min sample was not detected after administration of buffer solution or the 10 mM bile salt micellar solution, but a marked enhancement in plasma concentration was observed after administration of monoolein or the unsaturated fatty acid mixed micellar solution. Lauric acid and myristic acid had a small effect on the absorption. There was no increase of plasma concentration after administration of the mixed micellar solution composed of oleyl alcohol, methyl oleate, diolein, triolein, caprylic acid and palmitic acid.

Fig. 1 shows the time course of the disappearance of streptomycin and oleic acid from the large intestinal loop after administration of the mixed micellar solution. The absorption rate of oleic acid was fast during the first 15 min and after that became slow. The disappearance of streptomycin showed a similar pattern with oleic acid. Consequently, it is considered that the absorption of lipid may trigger the absorption of streptomycin.

To clarify the difference of the enhancing effects of monoolein and fatty acids, the absorbability of lipids, the interaction of streptomycin with mixed micelles and the alteration of the mucosal membrane permeability were examined. Table 3 shows the disappearance of streptomycin and lipids from the large intestine for 1 h after administration of various mixed micellar solutions. Although about 50% or more of all lipids were absorbed, streptomycin was absorbed to a greater extent in the presence of monoolein or unsaturated fatty acid mixed micelles than saturated fatty acid mixed micelles. The difference of the enhancing effects was not attributed to the absorbability of lipids.

Tokunaga et al. suggested that the interaction of heparin with mixed micelles might

TABLE 2

PLASMA CONCENTRATION OF STREPTOMYCIN AT 15 MIN AFTER ADMINISTRATION OF 10 mM MIXED MICELLAR SOLUTION TO THE LARGE INTESTINE

Administered dose of streptomycin was 4 mg/200 g rat.

Composition	μg/ml ± S.E.M.		Composition	$C_{m:n}$	μg/ml ± S.E.M.	
None	<1.5	(4)	Caprylic acid	$C_{8:0}$	<1.5	(5)
10 mM NaTC	<1.5	(5)	Lauric acid	$C_{12:0}$	5.0 ± 1.7	(4)
Monoolein	14.2 ± 1.7	(5)	Myristic acid	$C_{14:0}$	3.4 ± 0.8	(4)
Diolein	<1.5	(5)	Palmitic acid	$C_{16:0}$	<1.5	(4)
Triolein	<1.5	(5)	Palmitoleic acid	$C_{16:1}$	7.7 ± 0.7	(3)
Oleyl alcohol	<1.5	(4)	Oleic acid	$C_{18:1}$	14.9 ± 2.1	(4)
Methyl oleate	<1.5	(5)	Linoleic acid	$C_{18:2}$	14.4 ± 1.3	(5)
			Linoleic acid	$C_{18:3}$	13.2 ± 1.0	(4)

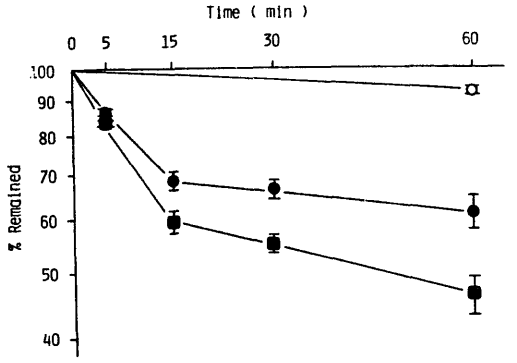

Fig. 1. Time course of the disappearance of streptomycin and oleic acid from the large intestine. Each value is the mean ± S.E.M. for 4–5 animals. ○, streptomycin after administration of buffer solution. ●, ■, streptomycin and oleic acid, respectively, after administration of 10 mM mixed micellar solution.

play a critical role in the enhancement of its absorption (Tokunaga et al., 1978). In this investigation, the interaction of streptomycin with mixed micelles was examined and the results are shown in Table 4. There was no correlation found between the interaction and the difference in the enhancing effects.

Effect of mixed micelles on the mucosal membrane permeability

To examine the alteration of the mucosal membrane permeability in the presence of mixed micelles, an exsorption experiment was done using sulfanilic acid which is almost impermeable to the intestinal membrane and the results are shown in Fig. 2. At the beginning, buffer solution was perfused into the large intestinal lumen, and then the perfusate was exchanged for 10 mM micellar or a mixed micellar solution after 20 or 40 min. At 60 min again the perfusate was changed to buffer solution. The exsorption of sulfanilic acid was very small during perfusion with buffer solution and the exsorption

TABLE 3

DISAPPEARANCE OF STREPTOMYCIN AND LIPID FROM THE LARGE INTESTINE FOR 1 h AFTER ADMINISTRATION OF 10 mM MIXED MICELLAR SOLUTION

Composition	% Disappeared ± S.E.M.		
	Streptomycin	Lipid	
None	7.0 ± 1.4	–	(4)
10 mM NaTC	13.5 ± 1.0	–	(4)
Monoolein	29.1 ± 2.4	53.6 ± 2.9	(4)
Lauric acid	17.1 ± 2.0	80.7 ± 1.7	(5)
Myristic acid	16.6 ± 3.0	52.7 ± 1.4	(4)
Palmitic acid	11.5 ± 1.0	68.0 ± 4.1	(5)
Palmitoleic acid	28.9 ± 2.3	53.4 ± 4.1	(4)
Oleic acid	38.5 ± 3.5	53.9 ± 3.1	(4)
Linoleic acid	34.4 ± 3.3	52.2 ± 3.1	(4)

TABLE 4

INTERACTION OF STREPTOMYCIN WITH MIXED MICELLES

Composition	% Binding
NaTC + lauric acid	61.6
NaTC + oleic acid	68.2
NaTC + linoleic acid	46.2
NaTC + monoolein	18.2

rate did not increase when the perfusate was changed to sodium taurocholate micellar solution (Fig. 2A). On the contrary, the exsorption rate increased when the perfusate was subsequently changed to monoolein–bile salt mixed micellar solution. Finally, when mixed micellar solution was replaced by buffer solution, the exsorption rate was rapidly reduced to the control level (Fig. 2A). In a similar manner, oleic acid and linoleic acid mixed micelles increased the exsorption of sulfanilic acid. The enhancement of the exsorption was less noticeable in the case of lauric or palmitic acid mixed micelles (Fig.

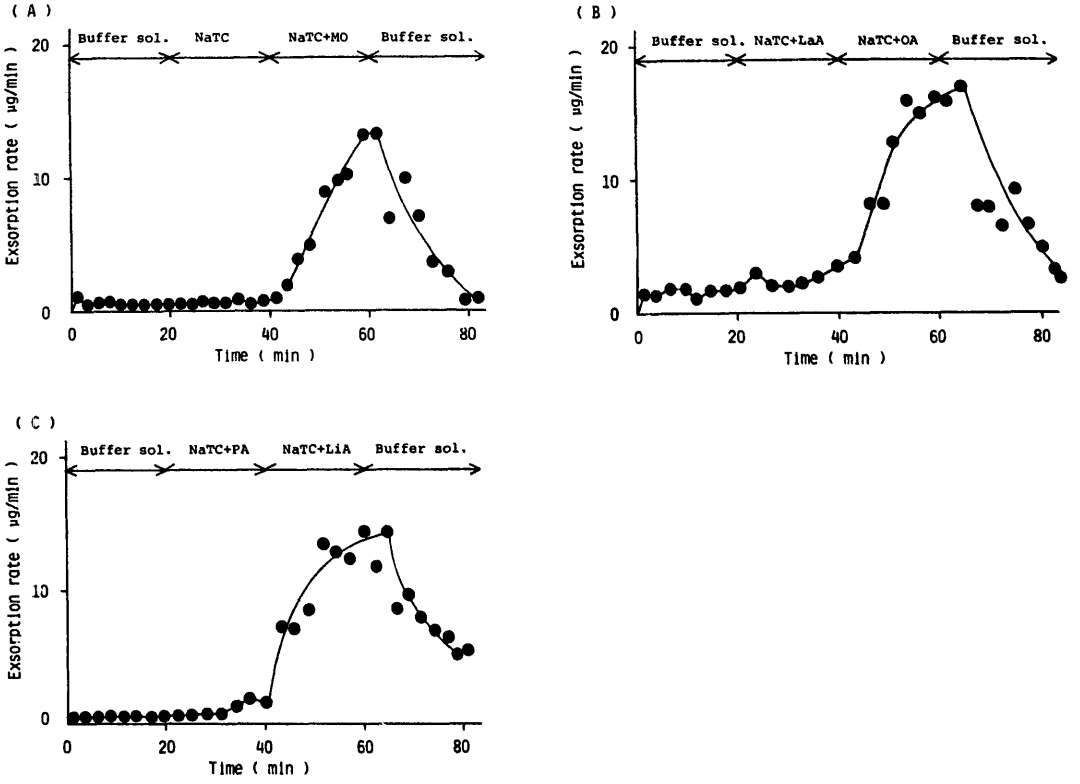

Fig. 2. Effect of mixed micellar solution on the exsorption of sulfanilic acid in the large intestine. A: NaTC = sodium taurocholate, MO = monoolein. B: LaA = lauric acid, OA = oleic acid. C: PA = palmitic acid, LiA = linoleic acid.

2B and C). A close correlation was found between the enhancing effect of mixed micelles on the intestinal drug absorption and the increase in the permeability of the mucosal membrane.

DISCUSSION

The effect of bile salts on the intestinal absorption of drugs has been investigated (Gibaldi, 1970; Kimura et al., 1972). Bile salts enhanced the absorption of poorly absorbed drugs such as phenol red (Feldman et al., 1970), sulfaguanidine (Kakemi et al., 1970) and urogastrone (Hori et al., 1977). In this investigation bile salt also caused an enhanced absorption of streptomycin to a small degree. On the other hand, monoolein– or unsaturated fatty acid–bile salt mixed micelles enhanced the absorption of streptomycin more than bile salt alone. Inui et al. investigated the effect of long-chain fatty acids on the absorption of water soluble drugs in sodium taurocholate solution (Inui et al., 1976). They reported that a significant increase in the absorption of procaine amide, a poorly absorbable drug, was observed in the presence of sodium taurocholate alone and a similar effect in the presence of oleic acid with sodium taurocholate was also found in the small intestine. The disagreement with our results in the enhancing effect of mixed micelles may be due to the difference of the concentration of mixed micelles.

The effect of bile salts on the function of the small (Russel et al., 1973; Teem and Phillips, 1972) and large intestine (Mekjian and Phillips, 1970, 1971) were studied and it was demonstrated that trihydroxy-bile salts such as sodium taurocholate were much less harmful to the intestinal tract in contrast to dihydroxy-bile salts. The following facts are known about lipid–bile salt mixed micelles. The addition of oleic acid or monoolein to a 10 mM solution of sodium taurodeoxycholate resulted in a protective effect on the everted intestinal membrane from the gross histological effect of the bile salt. The presence of taurocholate and fatty acid or monoolein within the intestinal lumen markedly modified a number of the toxic effects of deoxycholate on jejunal function (Lamabadusuriya et al., 1975). Taurocholic acid depressed amino acid absorption but the additon of monoolein restored amino acid absorption to normal (Dimagno et al., 1977). These findings suggest that mixed micelles may be a suitable and safe adjuvant to induce the intestinal absorption of poorly absorbed drugs.

Unsaturated fatty acids enhanced the intestinal absorption of streptomycin more than saturated fatty acids (Tables 1, 2 and 3). The lower the melting point of the fatty acid was, the more the drug absorption was increased in the presence of mixed micelles. Methyl oleate, oleyl alcohol, triolein and diolein which do not have a polar head did not increase the absorption. Therefore, it is suggested that lipids which have a polar head and a low melting carbon chain such as monoolein and oleic acid may enhance the intestinal absorption of poorly absorbable drugs when administered in the form of a bile salt mixed micellar solution.

It is interesting to note that lipids which enhanced the intestinal absorption of drugs are fusogenic lipids (Ahkong et al., 1973). Lucy et al. suggested that the presence of a low melting point fusogenic lipid might produce an increase in the permeability of the lipid bilayer (Maggio and Lucy, 1976). Exsorption experiments showed that the mucosal membrane permeability markedly increased in the presence of monoolein, oleic acid or linoleic

acid mixed micelles within the intestinal lumen, and the results of the previous pretreatment study showed that the alteration of the permeability was reversible. It was found that lipids such as monoolein or unsaturated fatty acids markedly enhanced the permeability of liposomal membranes, prepared from egg phosphatidylcholine, when incorporated into the membrane (the latter report). Based on the above-mentioned studies, it is considered that the enhanced intestinal absorption of drugs by the addition of mixed micelles is mostly due to the increase in the permeability of the mucosal membrane caused by the incorporation of the lipid component of mixed micelles.

REFERENCES

Ahkong, Q.F., Fisher, D., Tampion, W. and Lucy, J.A., The fusion of erythrocytes by fatty acids, esters, retinol and α-tocopherol. Biochem. J., 136 (1973) 147–155.

Dimagno, E.P., Malagelada, J.R. and Go, V.L.W., Effects of bile acids, lecithin and monoolein on amino acid absorption from the human duodenum. Proc. Soc. Exp. Biol. Med., 154 (1977) 325–330.

Duncombe, W.G., The colorimetric micro-determination of long-chain fatty acids. Biochem. J., 88 (1963) 7–10.

Faure, F. and Blanquet, P., Fluorometric microdetermination of streptomycin and dihydrostreptomycin by the reaction using β-naphthoquinone-4-sodium sulphonate in alkaline medium. Clin. Chim. Acta, 9 (1964) 292–300.

Feldman, S., Salvino, M. and Gibaldi, M., Physiologic surface-active agents and drug absorption. VII: Effect of sodium deoxycholate on phenol red absorption in the rat. J. Pharm. Sci., 59 (1970) 705–707.

Gibaldi, M., Role of surface-active agents in drug absorption. Fed. Proc., 29 (1970) 1343–1349.

Hori, R., Okumura, K., Inui, K., Nakamura, N., Miyoshi, A. and Suyama, T., Phamaceutical approach to the oral dosage form of macromolecules: effects of bile salts and oil-in-water emulsions on the intestinal absorption of urogastrone in the rat. Chem. Pharm. Bull., 25 (1977) 1974–1979.

Inui, K., Shintomi, M., Hori, R. and Sezaki, H., Intestinal absorption of drugs from solubilized systems of long-chain fatty acids in the rat. Chem. Pharm. Bull., 24 (1976) 2504–2508.

Kakemi, K., Sezaki, H., Konishi, R., Kimura, T. and Okita, A., Effect of bile salts on the gastrointestinal absorption of drugs. II. Mechanism of the enhancement of the intestinal absorption of sulfaguanidine by bile salts. Chem. Pharm. Bull., 18 (1970) 1034–1039.

Kimura, T., Sezaki, H. and Kakemi, K., Effect of bile salts on the gastrointestinal absorption of drugs. IV. Site of intestinal absorption of sodium taurocholate and its consequence on drug absorption in the rat. Chem. Pharm. Bull., 20 (1972) 1656–1662.

Lamabadusuriya, S.P., Guiraldes, E. and Harries, J.T., Influence of mixtures of taurocholate, fatty acids and monoolein on the toxic effects of deoxycholate in rat jejunum in vivo. Gastroenterology, 69 (1975) 463–469.

Maggio, B. and Lucy, J.A., Polar-group behaviour in mixed monolayers of phospholipids and fusogenic lipids. Biochem. J., 155 (1976) 353–364.

Mekhjian, H.S. and Phillips, S.F., Perfusion of the canine colon with unconjugated bile acids: effect on water and electrolyte transport, morphology and bile acid absorption. Gastroenterology, 59 (1970) 120–129.

Mekhjian, H.S., Phillips, S.F. and Hofmann, A.F., Colonic secretion of water and electrolytes induced by bile acids: perfusion studies in man. J. Clin. Invest., 50 (1971) 1569–1577.

Muranishi, S., Muranushi, N. and Sezaki, H., Improvement of absolute bioavailability of normally poorly absorbed drugs: inducement of the intestinal absorption of streptomycin and gentamicin by lipid–bile salt mixed micelles in rat and rabbit. Int. J. Pharm., 2 (1979) 101–111.

Norman, A., Preparation of conjugated bile acids using mixed carboxylic acid anhydrides. Ark. Kemi, 8 (1955) 331–342.

Russel, R.I., Allan, J.G., Gerskowitch, V.P. and Cochran, K.M., The effect of conjugated and unconjugated bile acids on water and electrolyte absorption in the human jejunum. Clin. Sci. Mol. Med., 45 (1973) 301–311.

Teem, M.V. and Phillips, S.F., Perfusion of the hamster jejunum with conjugated and unconjugated bile acids: inhibition of water absorption and effects on morphology. Gastroenterology, 62 (1972) 261–267.

Tokunaga, Y., Muranishi, S. and Sezaki, H., Enhanced intestinal permeability to macromolecules. I. Effect of monoolein-bile salts mixed micelles on the small intestinal absorption of heparin. J. Pharm. Dyn., 1 (1978) 28–38.

van Handel, E., Suggested modifications of the microdetermination of triglycerides. Clin. Chem., 7 (1961) 249–251.