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# Permeation of bile acids across artificial lipid membranes and Caco-2 monolayers

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*Dedicated to Prof. Dr. G. Zessin, Halle (Saale), on the occasion of his 65th birthday*

The permeation behavior of free bile acids (BA) with different numbers of hydroxyl groups as well as of the glycine and taurine conjugates was studied *in vitro* using artificial lipid membranes and Caco-2 monolayers. The transport across artificial lipid membranes depends strongly on the lipophilicity of the BA applied. Free BA permeated to a higher degree than the conjugated derivatives. The taurine conjugate was transported only to a very low extent. A distinct influence of the pH value in the donor medium on the permeation of the BA was observed. The transport rate decreased with increasing pH. Using Caco-2 monolayers, it was found that only unconjugated BA could permeate to a higher extent.

## 1. Introduction

Transport, permeation and absorption of drugs are influenced by food and food components (for instance in presence of dietary fibre preparations) [1–5] as well as by the properties of the drugs and by mixed micelle systems present in the small intestine.

Bile acids (BA) are released from the gallbladder in conjugated from (glycine/taurine ratio of ca. 3 : 1) into the duodenum. After emulsification and digestion of the dietary lipids, the BA can be reabsorbed via passive transport in the small intestine and the colon (free BA) or via a $Na^+$-coupled active transport system in the ileum [6–9].

Interactions of drugs and food components and their influence on drug absorption can be investigated using different *in vitro* methods. The interactions between the drugs and different mixed micelles, formed in the presence of BA, were determined by capillary electrophoresis [10–13]. BA were able to form extraordinarily stable mixed micelles with lipids (fatty acids and phospholipids) which may strongly influence drug absorption [12]. Different model systems using artificial or natural membranes have been developed and applied for the investigation of the transport of drugs [14]. For instance, it has been shown that BA influence the transport of propranolol across artificial lipid membranes and guinea pig mucosa alone or in presence of the dietary fibre pectin. Besides the structural and physico-chemical parameters of the polysaccharides, the structure of BA and their concentration in the system (above or below the critical micellar concentration) influence drug permeation [15].

Furthermore, different intestinal cell culture models have been proposed for the analysis of drug transport [16]. Caco-2 monolayers were intensively used in the last years for the prediction of the drug transport [17]. However, there are only a few informations about the transport of BA or about the influence of BA on the passive transport of drugs across artificial lipid membranes. It was found that the co-administration of chenodeoxycholic acid (CDC) was able to enhance the permeation of octreotide across Caco-2 monolayers [18]. On the other hand, the active absorption of BA in the small intestine was inhibited by cyclosporin A [19].

BA play an important role in the absorption of lipophilic drugs. These drugs are solubilized in stable mixed micelles formed by BA, phospholipids and/or fatty acids [12, 13]. On the other hand, BA were used in the last years as carrier molecules for drugs which are not or just to a negligible degree absorbed, respectively [20]. In this study, the permeation behavior of BA across artificial lipid membranes and Caco-2 monolayers was investigated as a function of the BA structure.

## 2. Investigations and results

The artificial collodium-dodecanol membrane have previously been used as a model for the transport and the absorption of drugs [3–5, 14]. Caco-2 monolayers are a well characterised transport model system [21] and seem to be suitable for the investigation of interactions of drugs and food ingredients or BA with a good correlation to the human intestinal epithelium.

Because of the pH value present in colon, our experiments with free BA were carried out in the pH range $\geq$6.0. The adjusted pH values were not changed during the transport experiments with both artificial membranes and Caco-2 monolayers.

The transport experiment across artificial lipid membranes showed that the permeation rate of the BA is strongly decreased with increasing pH values in the medium. This effect was found for all the tested BA (Figs. 1 and 2). The glycine conjugates of both dihydroxy BA used permeated to a higher extent and with almost the same rate. However, glycocholic acid, a derivative of a trihydroxy BA, was transported to a lower extent across the dodecanol membrane. On the other hand, taurine conjugated BA permeated only to a very small extent under the used conditions.

Free BA were transported across the artificial lipid membranes to a higher extent than the corresponding glycoconjugates. This effect was found for both the primary and the secondary BA at pH 6.0 and 8.0, respectively. At higher pH values, increased permeation rates were measured for the dihydroxy BA in comparison with cholic acid.

The concentration of BA in the donor compartment was changed slightly in the experiments with the Caco-2 monolayers (Fig. 3). Only in the case of CDC a distinct decrease in BA concentration was measured.

With the permeation time, the amount of BA increased in the acceptor compartment. The most distinct extent in the

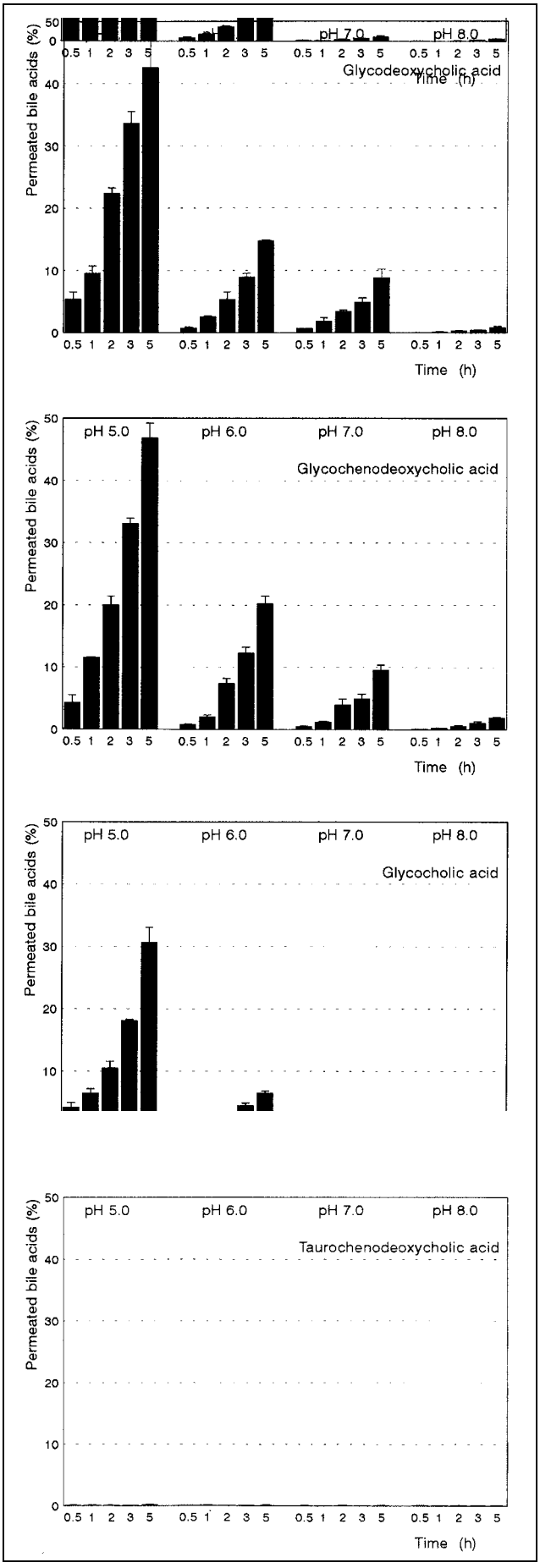


Fig. 1: Effect of pH and incubation time on the permeation of conjugated bile acids across artificial lipid membranes (Mean ±S.D.; n = 3)

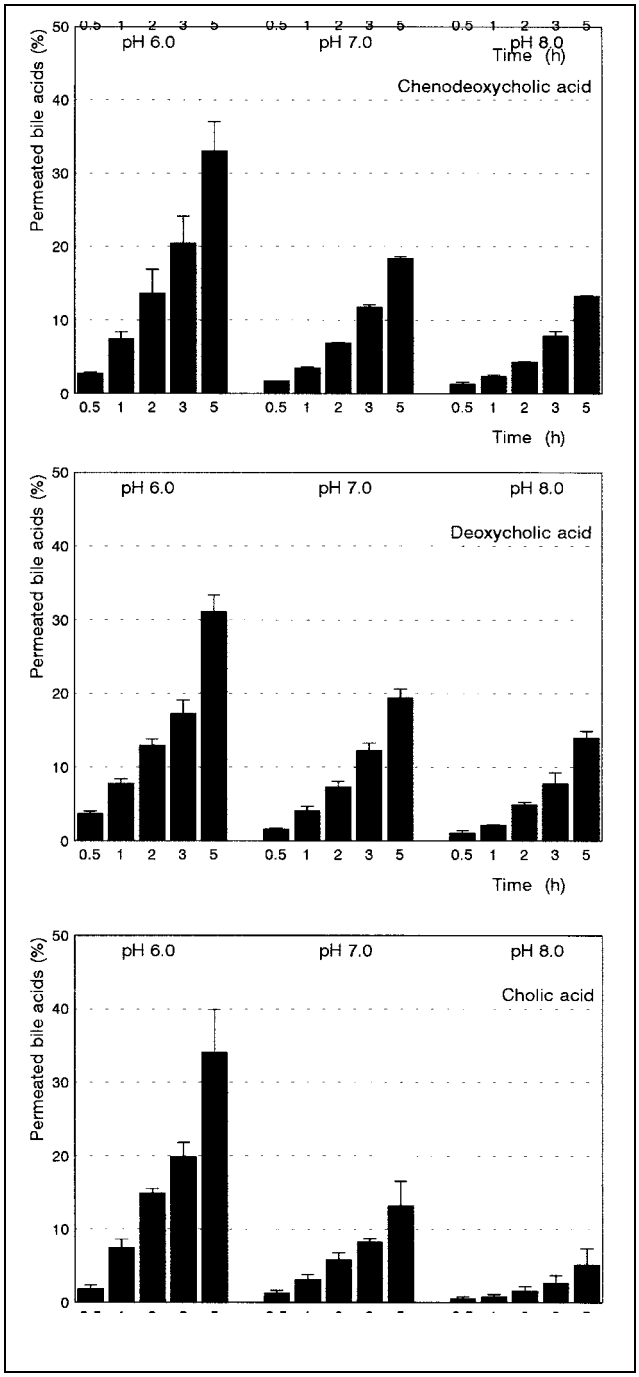


Fig. 2: Effect of pH and incubation time on the permeation of free bile acids across artificial lipid membranes (Mean ±S.D.; n = 3)

transport across the monolayer was found for the used free bile acid CDC. After 2 h, approximately 4.1% of this BA have been permeated. In the experiments using the artificial lipid membranes, 6.8% (pH 7) and 4.2% (pH 8)

**Table: Linear gradient fluids with acetonitril, methanol and water**

| Time (min) | A (%) | B (%) | C (%) | Flow (ml/min) |
|---|---|---|---|---|
| 0 | 30 | 40 | 30 | 1.0 |
| 50.0 | 69 | 5 | 26 | 1.0 |
| 50.1 | 100 | 0 | 0 | 1.0 |
| 58.0 | 100 | 0 | 0 | 1.5 |
| 63.0 | 30 | 40 | 30 | 1.0 |

A: acetonitril, B: methanol, C: water (% v/v/v)

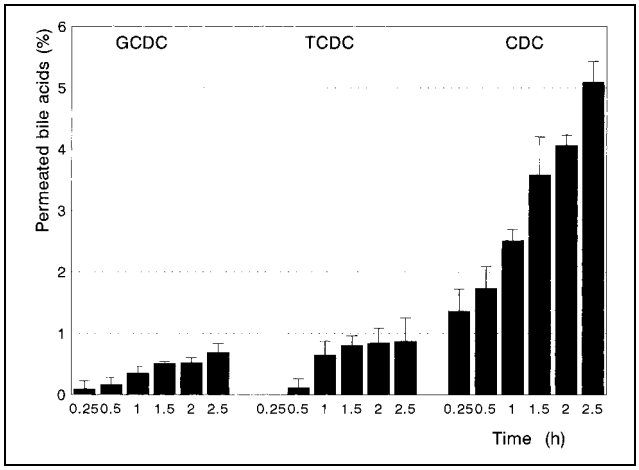


Fig. 3: Permeation of bile acids across Caco-2 monolayers at pH 6.8 (GCDC = glycochenodeoxycholic acid; TCDC = taurochenodeoxycholic acid; CDC = chenodeoxycholic acid; Mean ±S.D.; n = 3)

of the glycodeoxycholic acid were present in the acceptor compartment after 3 h (Fig. 2). Both of the conjugated BA permeated distinctly slower across the Caco-2 monolayers than the free BA. After 3 h, only approximately 0.8% of the glycochenodeoxycholic acid and 1.0% of the taurochenodeoxycholic acid were found in the acceptor compartment. These values are lower in the case of the taurine conjugate but higher for the glycine conjugate compared to the transport across the artificial lipid membrane.

## 3. Discussion

It was shown that structural parameters of the BA play a distinct role for their ability to pass artificial membranes and Caco-2 monolayers. The reason for this effect is the alteration of physico-chemical and functional properties of the BA by small structural changes.

In the lower parts of the ileum and especially in the colon, BA conjugated originally with glycine or taurine were deconjugated and then partly dehydroxylated by enzymes of the gastrointestinal microflora. As a result, free primary and secondary BA appear in the colon. Secondary BA have in contrast to the primary BA no hydroxyl group at the position 7 of the cholanoic acid skeleton.

The differences in the hydrophilic/hydrophobic properties of the BA caused by the structure of the steroid nucleus (number, position and orientation of hydroxyl groups, conjugation) play an essential role in their transport properties. Generally, the secondary BA are more lipophilic and they are better surfactants than the corresponding primary BA.

The lipophilicity was higher when unconjugated BA were present in acidic form than in the ionized form. Furthermore, the degree of dissociation is of substantial importance for the transport and absorption of BA.

It is known that the hydroxyl groups and the conjugation of the carboxyl groups inhibit the passive transport of BA. But they are important for the active absorption of BA *in vivo* [22]. Hydrophobic BA are absorbed at a higher rate by passive non-ionic absorption than the hydrophilic BA [23]. It was shown [22, 24–25] that conjugation prevents passive absorption of these amphiphilic detergent-like molecules across lipid bilayers unless an active carrier mechanism is present. Amelsberg et al. [26] found no significant differences in the absorption rates between glycine and taurine conjugates across the perfused guinea pig jejunum. The results obtained in this study show that the unconjugated BA, in contrast to the conjugated BA, do passively permeate through artificial lipid membranes. Therefore, passive absorption from the gastrointestinal tract appears to play an important role for the unconjugated BA. On the other hand, the conjugated BA are transported across the Caco-2 cells, however, to a low extent (about 1% in 2.5 h). As described in the literature these BA appear to be actively absorbed.

Unconjugated BA such as CDC are also transported to a high extent across Caco-2 cells. Both passive and active transport mechanisms appear to be important for the absorption of unconjugated BA such as CDC.

The results obtained in the study show that mainly unconjugated BA are able to permeate across artificial lipid membranes and Caco-2 cells.

Furthermore, passive absorption appears to play an important role for unconjugated BA. The significance of this *in vitro* results for the transport and absorption of drugs needs to be investigated.

## 4. Experimental

### *4.1. Permeation model system*

The *in vitro* permeation model system [14] used, consisted of donor and acceptor compartments separated by am artificial lipid membrane with collodium as matrix and dodecanol as lipid. The experiments were carried out at pH 5.0, 6.0, 7.0 and 8.0 (Sörensen phosphate buffer; 0.067 M) and 37 °C in the presence of 1 mmol/l bile acids. The concentration of the permeated BA was measured in the acceptor compartment up to 5 h.

### *4.2. Caco-2 monolayer model system*

The Caco-2 cell line was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). The cells were cultured at 37 °C, 5% $CO_2$/95% air and 100% humidity in DMEM (Dulbeccos Mem) with 4.5 g/l D-glucose, 10% inactivated fetal bovine serum, 1% stable glutamine, 1% non-essential amino acids and penicillin/streptomycin (5000 U/5 mg/ml) (Seromed, Berlin, Germany). The medium was changed daily. The cells were used between passage 12 and 15. The cells were split 1 : 10 upon reaching 80% confluence which occurred approximately every sixth day and set up in either T75 flasks (Falcon, Heidelberg, Germany) for maintenance of stocks or on 12 mm snapwells, 0.4 μm pore size (Corning Costar Corporation, Bodenheim, Germany), for diffusion experiments. Snapwells consisted of a microporous membrane mounted in a removable ring which can be inserted into a diffusion chamber. The snapwells were coated for better adhesion of the cells with collagen typ 1 from calf (sigma C-9791). Cells set up on coated snapwells were plated at a density of $6.3 \times 10^4$ cells/cm$^2$ in 0.5 ml medium. The snapwells were placed in a well (with 2 ml medium) of six-well cell culture cluster plates under steril conditions.

The cell layer integrity has been checked by measuring of transepithelial electrical resistance (TEER) using the special resistent device ENDOHM and the Epithelial Voltohmmeter EVOM (World Precision Instruments, Berlin, Germany) every second day.

All cell layers on the snapwells used were confluent and reached a maximum of TEER after about 10–15 d. We used the cell coated snapwells for a diffusion experiment at day 19 or 20.

The diffusion chamber system from Corning Costar Corporation has been used. This system is equipped with a gas lift system to prevent "unstirred water layers" and produces a homogeneous circulation of the bathing fluids. The valves on the gas manifold were adjusted to give an uniform delivery of the gas mixture (5% $CO_2$, 95% $O_2$; Carbogen, Linde, Höllriegelskreuth, Germany) to each of the half-chambers. The snapwells with the cell monolayer were inserted into the diffusion chambers. The chambers were placed in the block heater, which was connected with a thermostatically controlled recirculating water bath to maintain a constant temperature of 37 °C. The transport experiments were started immediately by adding 5 ml medium with BA to the donor chamber and without BA to the acceptor chamber. The experiments were done in two parallel series in cultural media at pH 7.6 and 37 °C in the presence of 1 mmol/l BA in the apical-to-basolateral direction. The concentration of permeated BA was measured in the acceptor chamber up to 2.5 h.

### *4.3. Bile acids*

Glycochenodeoxycholic acid (GCDC), glycocholic acid, glycodeoxycholic acid and cholic acid were obtained from Sigma Chemical Co., St. Louis, USA. Taurochenodeoxycholic acid (TCDC) was purchased from Calbio-

chem Co., La Jolla, USA. Chenodeoxycholic acid and deoxycholic acid were obtained from Fluka Feinchemikalien GmbH, Neu-Ulm, Germany. All BA were of analytical grade.

### *4.4. Estimation of bile acids*

Their permeated BA solutions were purified by solid phase extraction on Bakerbond spe Octadecyl ($C_{18}$) columns in the Baker spe-12G system (J. T. Baker, Groß-Gerau, Germany) activated previously with 5 ml methanol and then with 10 ml water. After adsorption of the BA on the column and washing with 20 ml water, the BA were eluted with 2 ml MeOH and then dried in a vacuum centrifugal evaporator (Jouan GmbH, Unterhaching, Germany).

The extracted BA were estimated by HPLC using pre-column derivatization and fluorescence detection [27]. Free and glycine conjugated BA were directly derivatized with 4-bromomethyl-7-methoxycoumarin (BMC) in the presence of 18-Crown-6 as a catalyst. Taurine conjugates, which did not react with BMC, were enzymatically hydrolysed with cholylglycine hydrolase before derivatization and analysis as free bile acids.

The BMC labelled derivatives were analysed on a non-polar stationary phase (Nucleosil 100 Å; $C_{18}$; 5 µm; 250 × 4.6 mm) at 40 °C in HPLC equipment from Gynkotek (Germering, Germany) with online-degasser DG 1310, gradient pump M 480, injection automat GINA 160, column oven (Peltier), fluorescence detector RF 1002 (excitation 320 nm; emission 385 nm) (Shimadzu Europe, Duisburg, Germany) and GynkoSoft software.

Linear gradients consisting of acetonitril, methanol and water were applied according to the schedule presented in the Table.

**References**


1 Welling, P. G.; in: D'Arcy, P. F.; McElnay, J. C.; Welling, P. G. (Eds.): Mechanisms of Drug Interactions, p. 45, Springer, Berlin 1996
2 Neubert, R.; Fritzsch, B.; Dongowski, G.: Pharmazie **48**, 723 (1993)
3 Neubert, R.; Fritzsch, B.; Dongowski, G.: Pharmazie **50**, 414 (1995)
4 Dongowski, G.; Schnorrenberger, B.; Plätzer, M.; Schwarz, M.; Neubert, R.: Int. J. Pharm. **158**, 99 (1997)
5 Dongowski, G.; Neubert, R.; Schwarz, M.; Plätzer, M.; Schnorrenberger, B.; Anger, H.: Pharmazie **53**, 871 (1998)
6 Marcus, S. N.; Schteingart, C. D.; Marquez, M. L.; Hofmann, A. F.; Xia, Y.; Steinbach, J. H.; Ton-Nu, H. T.; Lilienau, J.; Angellotti, M. A.; Schmassmann, A.: Gastroenterology **100**, 212 (1991)
7 Aldini, R.; Roda, A.; Lenzi, P. L.; Ussia, G.; Vaccari, M. C.; Mazzella, G.; Festi, D.; Bazzoli, F.; Galletti, G.; Casanova, S.; Montagnani, M.; Roda, M.: Eur. J. Clin. Invest. **22**, 744 (1992)
8 Aldini, R.; Roda, A.; Montagnani, M.; Roda, E.: Ital. J. Gastroenterol. **27**, 141 (1995)
9 Stedronsky, E. R.: Biochim. Biophys. Acta **1210**, 255 (1994)
10 Schwarz, M. A.; Neubert, R. H. H.; Rüttinger, H. H.: J. Chromatogr. A **745**, 135 (1996)
11 Schwarz, M. A., Neubert, R. H. H.; Dongowski, G.: Pharm. Res. **13**, 1174 (1996)
12 Schwarz, M. A.; Raith, K.; Rüttinger, H. H.; Dongowski, G.; Neubert, R. H. H.: J. Chromatogr. A **781**, 377 (1997)
13 Schwarz, M. A.; Raith, K.; Dongowski, G.; Neubert, R. H. H.: J. Chromatogr. A **809**, 219 (1998)
14 Neubert, R.: Pharmazie **45**, 233 (1990)
15 Dongowski, G.; Neubert, R.; Haase, H.; Schnorrenberger, B.: Int. J. Pharm. **144**, 233 (1996)
16 Quaroni, A.; Hochman, J.: Adv. Drug Deliv. Rev. **22**, 3 (1996)
17 Artursson, P.; Palm, K.; Luthman, K.: Adv. Drug Deliv. Rev. **22**, 67 (1996)
18 Fricker, G.; Fahr, A.; Beglinger, C.; Kissel, T.; Reiter, G.; Drews, J.: Br. J. Pharmacol. **117**, 217 (1996)
19 Sauer, P.; Klöters-Plachky, P.; Stiehl, A.: J. Clin. Invest. **25**, 677 (1995)
20 Swaan, P. W.; Szoka, F. C.; Øie, S.: Adv. Drug Deliv. Rev. **20**, 59 (1996)
21 Dix, C. J.; Hassan, I. F.; Obrary, H. Y.; Shah, R.; Wilson, G.: Gastroenterology **98**, 1272 (1990)
22 Aldini, R.; Montagnani, M.; Roda, A.; Hrelia, S.; Biagi, P. L.; Roda, E.: Gastroenterology **110**, 459 (1996)
23 Crosignani, A.; Setchell, K. D. R.; Invernizzi, P.; Larghi, A.; Rodrigues, C. M. P.; Podda, M.: Clin. Pharmacokinet. **30**, 333 (1996)
24 Hofmann, A. F.; in: Johnson, L. R. (Ed.): Physiology of the Gastrointestinal Tract, 3. Ed. p. 1555, Raven Press, New York 1994
25 Roda, A.; Gioacchini, A. M.; Manetta, A. C.; Cerrè, C.; Montagnini, M.; Fini, A.: Ital. J. Gastroenterol. **27**, 327 (1995)
26 Amelsberg, A.; Schteingart, C. D.; Ton-Nu, H.-T.; Hofman, A. F.: Gastroenterology **110**, 1098 (1996)
27 Wang, G.; Stacy, N. H.; Earl, J.: Biomed. Chromatogr. **4**, 136 (1990)


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