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INHIBITION OF COLCHICINE ABSORPTION BY THE FAT SUBSTITUTES, SUCROSE POLYESTER AND TRICARBALLYLATE TRIESTER, IN THE RAT

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Summary

The effect of non-absorbable fat substitutes (sucrose polyester (SPE) and tricarballylate triester (TCTE)) on the enterohepatic circulation of colchicine was studied in the rat. In a first experiment, emulsions of either sunflower oil (SFO), SPE, or TCTE, were introduced into the ligated small intestine and compared to a control group receiving physiological saline. All the groups received colchicine as an intravenous bolus. The plasma levels of colchicine in all groups was not affected, and luminal samples indicated that SPE and TCTE have no influence on the biliary excretion of colchicine (a previous experiment in bile duct-cannulated rats showed that SPE and TCTE, introduced by intragastric tube, have no effect on bile flow rate). In a second experiment, colchicine diluted in bile was mixed with saline or emulsions of either SFO, SPE or TCTE, and introduced into the ligated small intestine. The area under the curve and the maximal plasma concentration of colchicine were reduced when the drug was mixed with SPE or TCTE rather than saline ($p < 0.0005$). After 150 min, luminal samples were taken and showed significantly higher ($p < 0.034$) concentrations of colchicine in both SPE and TCTE groups compared to the saline group, indicating a significant inhibition of reabsorption of biliary colchicine. In conclusion, the non-absorbable fat substitutes, SPE and TCTE, did not influence biliary excretion of colchicine but reduced its reabsorption, thus altering its enterohepatic circulation.

Key Words: fat substitutes, sucrose polyester, tricarballylate triester, colchicine

Ingestion of a non-absorbable fat substitute, sucrose polyester (SPE) (1), has been shown to reduce the reabsorption of cholesterol dissolved in bile both in rats and humans (2, 3). Volpenhein et al (4) suggested that the reduction in reabsorption by SPE could be extended to lipophilic compounds that are excreted in the bile. However, SPE has been shown to have no or minimal effect on bile acid excretion and absorption (3, 5). Since interruption of the enterohepatic circulation of a substance requires its transport from the aqueous biliary phase to the non-absorbed oily phase, the substance would have to be lipid soluble to be retained by the oily phase and thus not be reabsorbed.

In recent studies, we have shown that 50% of colchicine administered by the i.v. route is excreted in the bile (6), predominantly as the parent drug (7). Its partition coefficient is 2.52 (8), indicating that 71.6% of the drug is distributed in the lipidic phase. We therefore aimed to verify whether the non-absorbable fat substitutes, SPE and tricarballylate triester (TCTE), could have an influence on the enterohepatic circulation of colchicine (9). In this study, biliary excretion and intestinal reabsorption were separately investigated using *in situ* ligated intestine models in the rat.

Materials and Methods

Materials: Colchicine was from Fluka (Paris, France). All solutions were prepared in the dark. Polyethylene glycol (PEG 4000) was from Merck Clevenot (France). Radiolabelled Colchicine ([ring C, methoxy-^{3}H] colchicine, specific activity: 74.3 Ci/mmol; purity: 99%) and PEG ([1,2 ^{14}C]PEG, specific activity: 13.0 mCi/g) were obtained from New England Nuclear-Du pont de Nemours (Paris, France). Sunflower oil (SFO) was from Lesieur (France). SPE consisted of hepta- and octaesters of sucrose prepared with sunflower oil (Lipochim, Marseille, France). TCTE was prepared with tricarballylic acid and sunflower fatty alcohols (Lipochim, Marseille, France). Egg lecithin (lipoid E80) was from Seppic (Paris, France). Sodium pentobarbital (60 μg/ml) was from Sanofi (Libourne, France) and lipase from Sigma (Saint-Quentin-Fallavier, France). All other chemicals were of analytical grade.

Emulsion Preparation: All emulsions were prepared with 20 % (v/v) of either SFO, SPE, or TCTE in physiological saline. Each oil was mixed with the aqueous solution using a bladed stirrer and then sonicated (Labsonic 2000, B. Braun ScienceTec, Les Ulis, France). Emulsion stability was ensured by 1.2% (w/v) egg lecithin. The surface-active agent was previously dissolved in the lipid phase at 70°C. The diameter and dispersion of the lipid particles in the emulsions were checked with an autodilute submicron particle sizer (Nicomp 370, Hiac/Royco, Palaiseau, France).

Partition Coefficient Determination: The partition coefficient of colchicine between the lipid (SFO, SPE or TCTE) and aqueous (physiological saline) phases was determined at 37°C by shaking 2.0 ml of the lipid phase and 2.0 ml of the aqueous solution containing colchicine at a concentration equal to that used in the experiments. After centrifugation, colchicine concentration in the aqueous phase was determined by measuring [^{3}H]colchicine radioactivity. The partition coefficient (P) was calculated from the ratio C_{lip}/C_{aq}, where C_{aq} is the concentration in the aqueous phase after centrifugation, and C_{lip} the colchicine concentration in the lipid phase calculated by substraction of C_{aq} from the initial concentration of colchicine in the aqueous phase.

Experiments: Sprague Dawley male rats (Iffa Credo, Lyon, France) weighing 280-300 g were fasted with free access to water for 16 to 20 hours before experimentation. The rats were anaesthetized by i.p. injection of sodium pentobarbital (55 mg/kg). To prevent hypothermic alteration in biliary excretion (10), body temperature was maintained at 37 ± 1°C by a heating lamp and monitored by a rectal probe (Ellab Thermometer model TE 3, Copenhagen, Denmark). All experiments were performed between 10 a.m. and 6 p.m.

Bile Flow Measurement: After a median laparatomy, the common bile duct was cannulated with polyethylene tubing (i.d. 0.3 mm, Biotrol, Paris, France). The abdomen was then closed. After 30 minutes, the rats were given 1 mL of either physiological saline or emulsions of SFO, SPE, or TCTE, administred using a soft intragastric feeding tube with syringe. Bile samples were collected in preweighed tubes at 0-30, 30-60, 60-90, 90-120, 120-150, and 150-180 min. The volume of bile was determined gravimetrically.

Enterohepatic Circulation of Colchicine:

1. Biliary Excretion: Femoral vein and artery were cannulated with polyethylene tubing (i.d. 0.38 mm, Biotrol, Paris, France) for drug administration and blood sampling, respectively. The whole length of the small intestine was filled with 5 mL of either physiological saline or emulsions of SFO, SPE, or TCTE containing 5 mg/mL [^{14}C]PEG (4.5 μCi/mg) as a non-absorbable marker to verify any transfer of water into or out of the intestinal lumen (11). Loss of liquids was prevented by silk ligatures at the gastric and caecal ends. Immediately afterwards, the animals received a single i.v. bolus dose of 15 μg/kg (2.5 μCi/kg) [^{3}H]colchicine prepared in physiological saline. After drug adminstration, blood samples (0.4 mL) were collected at 2, 5, 15, 30, 60, 90, 120, and 150 min in heparinized tubes. After each blood sampling, an equivalent volume of saline was administered, and the arterial cannula was filled with saline. Samples were immediately centrifuged and the plasma frozen until analysis. At 150 min, luminal samples were taken, centrifuged and the supernatant frozen until analysis.

2. Intestinal Reabsorption: The intestine was ligated 4-5 cm distant from the pylorus to avoid *de novo* biliary excretion of colchicine in the luminal fluid. The second ligature was set at the caecal end. The bile samples collected previously (during the bile flow measurement experiment) were pooled for each donor rat and homogenized. The dose administered was 600 μL/100 g (4 μL/min/100 g x 150 min). Fifty percent of the dose of colchicine administered in the first experiment (0.75 μg/100 g) with the same radioactivity (0.25 μCi/100 g) was mixed with the bile samples. These were then added to either saline or emulsions of SFO, SPE, or TCTE containing [^{14}C]PEG and 0.6% (w/v) lipase (this was added to compensate for the non-secretion of pancreatic lipase in the intestinal segment). Five mL of these solutions was administered into the intestinal segment. The mesenteric vein was cannulated for blood sampling performed at the same times as above, the first being performed 5 min after administration. At 150 min, luminal samples were taken.

Analytical Methods: Fifty microliter aliquots of plasma or luminal samples (in duplicate) were mixed with 3 ml Pico-Fluor 40$^{(R)}$ scintillation liquid (Packard, Rungis, France) in a minivial. Radioactivity was measured by liquid scintillation counting with a beta-counter (Tri-Carb 1900 TR, Packard, les Ulis, France) using automatic external standardization for the quenching correction. Total radioactivity was expressed as colchicine equivalent since it includes unchanged colchicine and its metabolites.

Data Analysis: ^{3}H radioactivity results were converted to drug concentrations and corrected for water movements measured by [^{14}C]PEG as described elsewhere (12). In the biliary excretion experiment, the colchicine concentrations measured in luminal samples were corrected for *i*) the additional volume of secreted bile (estimated from the bile flow measurement experiment) and *ii*) the reabsorption of biliary colchicine (estimated from the intestinal reabsorption experiment) as follows: $C_{(v)} = /C_{(v')} \cdot v'/v/ + C_{abs(v)}$ (I), where C is colchicine concentration in v the volume of luminal fluid (i.e. 5 mL) or $v'=v$ plus volume of secreted bile, and C_{abs} the concentration of biliary colchicine reabsorbed. The fraction of biliary excreted drug (f_B) was calculated. In the intestinal reabsorption experiment, the fraction of colchicine reabsorbed (f_R) was determined by substracting colchicine concentration measured in luminal samples at 150 min from the initial administered concentration.

Pharmacokinetic Analysis: Plasma concentration-time data from individual animals were analysed using a model-independent method. Estimates of elimination rate constant (β) were obtained by fitting the concentration-time data using the SIPHAR software (Simed, Creteil, France). The half-life ($t^1/_{2\beta}$) was calulated as 0.693/β. The area under the curve (AUC_{0-150}) was calculated by the trapezoidal method and the extrapolated $AUC_{150-\infty}$ was calculated by dividing the concentration of colchicine at 150 min by β. Clearance was determined as $Cl = dose/AUC_{0-\infty}$ and volume of distribution as $Vd_\beta = Dose/\beta.AUC_{0-\infty}$ for i.v. colchicine. Following oral administration, the maximal plasma concentration (Cmax) and the corresponding time (Tmax) were deduced from the experimental data. The colchicine bioavailability was determined by the equation $F = (AUC_{oral}/AUC_{i.v.}).(Dose_{i.v.}/Dose_{oral})$ using the experimental AUC (*i.e.* AUC_{0-150}).

Statistical Analysis: Results are expressed as mean ± SEM. Statistical analysis was performed using analysis of variance, and significance was set at $p < 0.05$.

Results

Emulsion oil particle diameters ranged from 115 to 565 nm ($p=0.95$) and were stable during storage at 4°C for at least one week. The colchicine partition coefficient with SFO was 0.74 ± 0.03 (mean ± SEM, n=4). The difference was significant compared to TCTE (0.99 ± 0.03, $p<0.0009$) or SPE (1.2 ± 0.11, $p<0.006$). There were no significant differences between TCTE and SPE ($p<0.6$).

Figure 1 shows steady-state bile flow rates *versus* time for all the treated groups. Mean flow rates did not show significant differences ($p<0.08$) between saline (41.5 ± 3.7), SFO (33.2 ± 2.3), SPE (41.9 ± 3.4), or TCTE (42 ± 4.2 μL/min/kg).

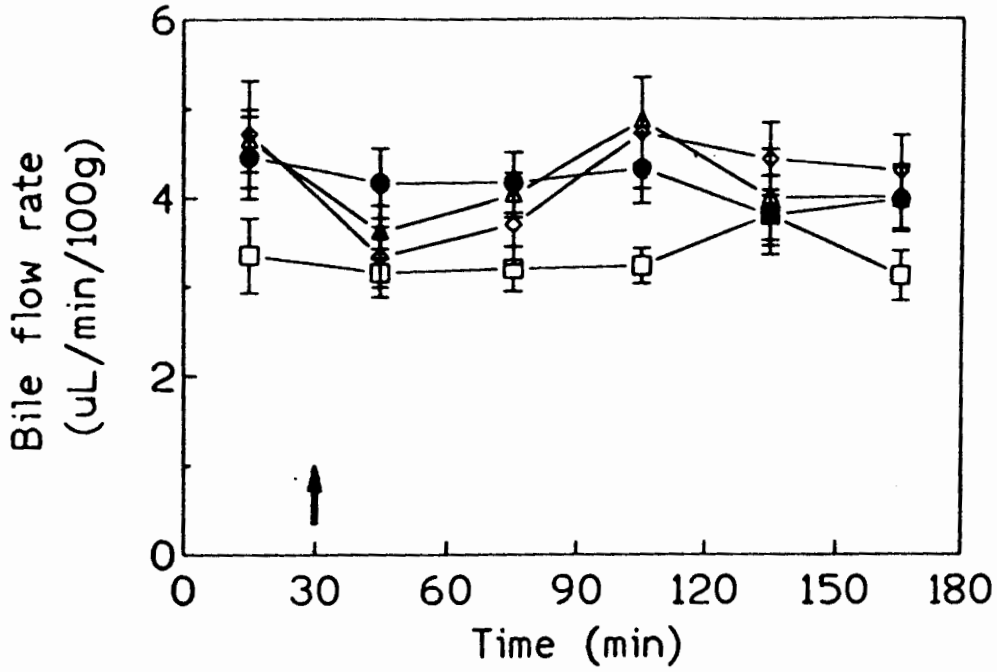

Fig. 1

Bile flow rate measurements after intragastric administration (↑) of saline (●), or emulsion of either SFO (□), TCTE (◇), or SPE (△). Results are expressed as mean for 6 rats. Vertical lines show SEM.

In the enterohepatic circulation experiments, [^{14}C]PEG measurements showed non-significant variations in PEG concentrations, indicating that water transfers were minor. Consequently, the decrease in colchicine concentration in the lumen was considered to be due to intestinal reabsorption. When the effect of the fat substitutes, SPE and TCTE, on the biliary excretion of colchicine was studied in comparison with saline and SFO, we saw no difference in plasma kinetics of colchicine administered by the i.v. route and no effect on the amount of colchicine excreted in bile as calculated according to formula (I) (Fig. 2 and Table I). Pharmacokinetic parameters were not significantly different between any of the groups, though SPE showed a 20% reduction in AUC compared to saline ($p < 0.14$).

TABLE I

Pharmacokinetic Parameters of [^{3}H]Colchicine After i.v. Administration, the Small Intestine Being Filled With Saline, or Emulsion of SFO, SPE, or TCTE. The Results are Expressed as Mean ± SEM (n=6). Statistics: Not Significant For All Groups.

Parameters	Saline	SFO	TCTE	SPE
$AUC_{0-\infty}$ (ng.mL^{-1}.min)	485 ± 57	472 ± 68	499 ± 62	386 ± 26
$t^1/_2\beta$ (min)	51 ± 10.5	64.3 ± 9.5	76.6 ± 26.4	56.6 ± 13.7
Vd_β (mL.kg^{-1})	1487 ± 296	1876 ± 308	1809 ± 456	1768 ± 367
CL (mL.min^{-1}.kg)	31.6 ± 3.3	32.9 ± 4.2	30.9 ± 3.2	36.9 ± 1.9
f_β (% dose)	53 ± 4.4	48 ± 3	45 ± 6.1	54 ± 1

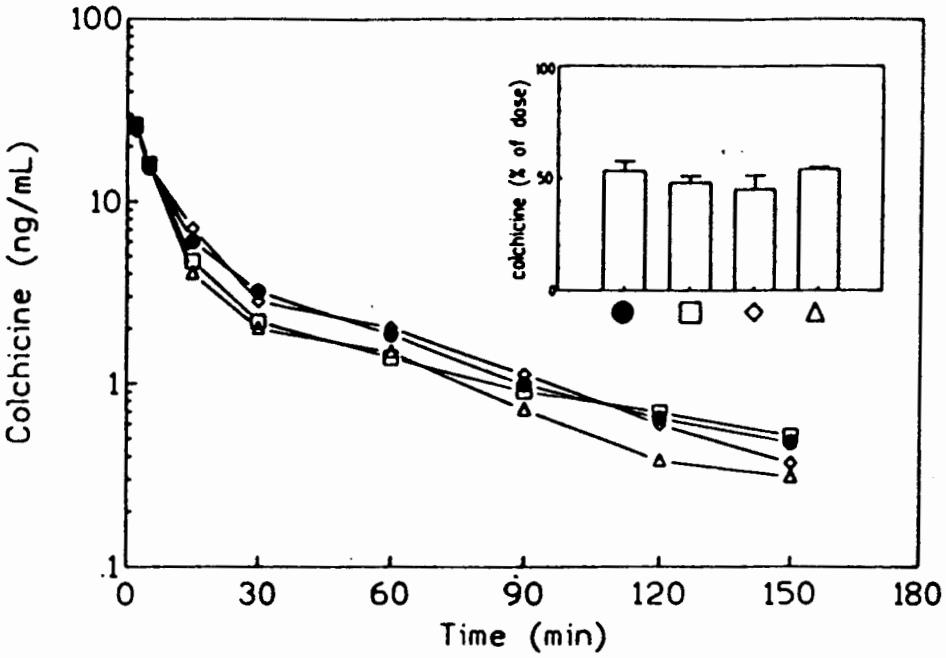

Fig. 2

Plasma concentration of [^{3}H]colchicine administered by i.v. route, the small intestine being filled with saline (●), or emulsion of either SFO (□), TCTE (◇) or SPE (△). Each point represents the mean for 6 rats. Histograms show the percentage of colchicine excreted in bile (mean ± SEM).

TABLE II

Pharmacokinetic Parameters of [^{3}H]Colchicine After Intra-intestinal Administration in Saline or in Emulsion with SFO, SPE, or TCTE. The Results Are Expressed as Mean ± SEM (n=6). Statistics: *: $p<0.05$ Compared to the NaCl-treated Group. +: $p<0.05$ Compared to the SFO-treated Group.

Parameters	Saline	SFO	TCTE	SPE
$AUC_{0-\infty}$ (ng.mL^{-1}.min)	110.3 ± 5	81.4 ± 3*	79.9 ± 2*	75.3 ± 0.8*+
C_{max} (pg/mL)	915 ± 23	671 ± 139	485 ± 83*	360 ± 0.2*+
t_{max} (min)	30	30	30	30
$t^1/_{2\beta}$ (min)	82.8 ± 12.6	79.4 ± 6.8	100.4 ± 12	111.4 ± 2.2*+
F (%)	35.6 ± 5.3	29 ± 5	23 ± 4	21.7 ± 1.7*
f_R (% dose)	42 ± 2.3	33 ± 14	21 ± 8.3*	19 ± 2.7*

Figure 3 shows plasma colchicine kinetics and the percentage of colchicine remaining in the lumen in the intestinal reabsorption experiment. After 150 min, SPE- and TCTE-treated groups showed significantly higher ($p<0.03$) concentrations of colchicine remaining in the lumen, compared to the saline-treated group, indicating minor reabsorption of biliary colchicine as shown in Figure 3 and in Table 2. All lipid-treated groups showed significantly ($p<0.0006$) lower AUCs but only SPE and TCTE reduced C_{max} significantly ($p<0.0005$) compared to the saline-treated group. Colchicine bioavailability was reduced by both lipids but the difference was

not significant ($p < 0.27$) compared to saline. On the other hand, the fraction of reabsorbed colchicine was significantly reduced by both SPE and TCTE compared to saline ($p < 0.03$).

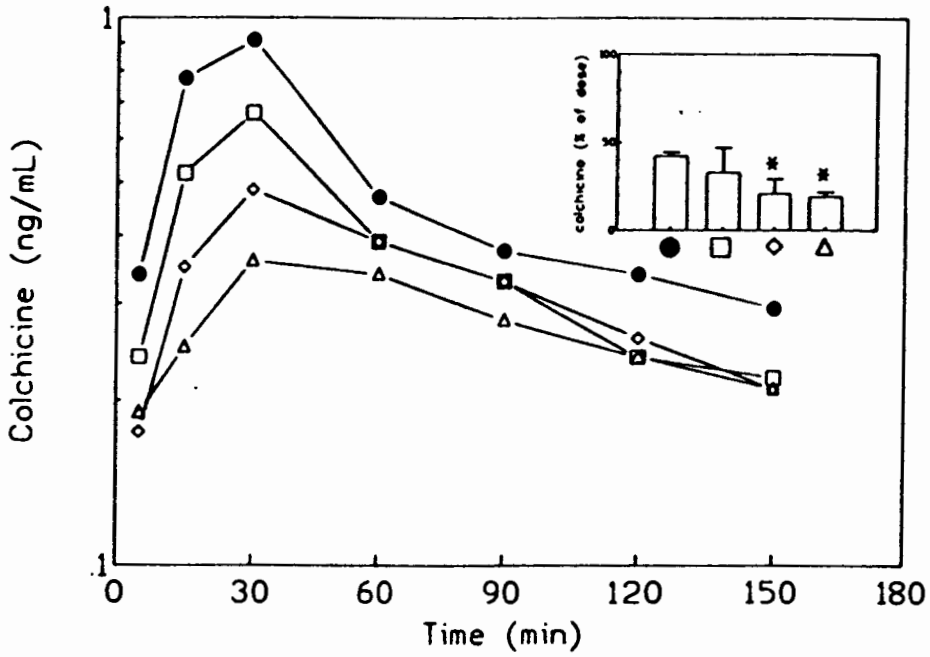

Fig. 3

Plasma concentrations of biliary [^{3}H]colchicine administered intra-intestinally in saline (●) or emulsion of either SFO (□), TCTE (◇) or SPE (△). Points represent mean for 6 rats. Histograms show the percentage of reabsorption of colchicine (mean ± SEM). Statistics: *: $p < 0.05$ compared to the saline-treated group.

Discussion

Colchicine is known to be extensively excreted via the biliary route mainly in the unchanged form and to be reabsorbed in the intestine (9). Consequently, the effect of fat substitutes, SPE and TCTE, which have been shown to alter digitoxin (12) and cyclosporine (13) absorption, could be evaluated in terms of *i*) effect on the biliary flow rate, *ii*) effect on biliary colchicine excretion, and *iii*) effect on colchicine reabsorption using different rat ligated intestine models. Both experiments were compared to control groups receiving saline or a digestible dietary lipid (SFO).

Suprisingly, the SFO-treated group showed a lower bile flow rate compared to the other groups, but the difference was not significant ($p < 0.08$). Moreover, as this low bile flow rate was the same before treatment, as shown in Figure 1, SFO had no effect on bile flow. This result is in contradiction with those of Juste et al (14) who found that bile flow rate rose with dietary fat content in the pig. Moreover, SFO did not increase the bioavailability of colchicine compared to saline, in contrast to its effect on digitoxin (12) and that of olive oil on cyclosporine (13). One explanation could be the lower partition coefficient of colchicine with SFO. Another explanation could be that colchicine alters intestinal absorption of lipids (15, 16) and therefore could interfere with absorption of that part of it dissolved in lipid. Barnwell et al showed that colchicine also alters secretion of lipids into bile (17) without affecting bile flow at any time after treatment in the rat (18).

On the other hand, our results demonstrate a significant reduction in the reabsorption of biliary colchicine by SPE and TCTE, thus altering its enterohepatic circulation. The administration of bile acids is known to increase bile flow but using our intestinal reabsorption model, de novo bile excretion measured in bile duct-cannulated rats was comparable to the amount of donor bile (data not shown). The maximal plasma colchicine concentration was reduced by 61% after SPE and 47% after TCTE compared to the saline-treated group. This effect is certainly due to the sequestration of colchicine by the non-absorbed oil phase of the fat substitutes. SPE has been proposed as an alternative lipid-lowering agent without sacrificing the organoleptic properties of fat (19) and TCTE is also a low calorie replacement candidate for edible fats in food use (20). Both SPE and TCTE are undigested and unabsorbed in the gastrointestinal tract (1, 20, 21) and SPE is shown to reduce cholesterol (3) and DDT (22) absorption, this effect being linked to its non-absorbability. By the same mechanism, liquid paraffin, a poorly absorbed mineral oil, reduced DDT absorption in rats (23). Guar gum, a gel-forming dietary fiber, also increases fecal elimination of cholesterol as bile acids (24). Gums are used to produce fat-free foods and to reduce the fat content of a wide variety of formulated foods (25).

In conclusion, the present study demonstrates an inhibitory effect of SPE and TCTE on colchicine reabsorption. This effect can be attributed to the resistance of SPE and TCTE to hydrolysis and their subsequent non-absorption. Colchicine dissolved in the non-digested oil phase of the fat substitutes is consequently not available for absorption. This finding has two implications: first, colchicine absorption could be impaired by co-administration with fat substitutes and this should be borne in mind when planning dosage regimens (even if the animal model used here do not simulate absorptive interferences in man since anaesthesia is known to reduce intestinal motility); second, as the biliary route is the major route of colchicine elimination, fat substitutes could be effective for interrupting the enterohepatic circulation of colchicine in cases of overdosage.

References

1. F.H. MATTSON and G.A. NOLLEN, J. Nutr. 102 1171-1175 (1972).
2. R.J. JANDACEK, F.H. MATTSON, S. Mc NEELY, L. GALLON, R. YUNKER, and C.J. GLUECK, Am. J. Clin. Nutr. 33: 251-259 (1980).
3. F.H. MATTSON and R.J. JANDACEK, Lipids 20 273-277 (1985).
4. R.A. VOLPENHEIN, D.R. WEBB, and R.J. JANDACEK, J. Tox. Environ. Health 6 679-683 (1980).
5. R.J. JANDACEK, Drug Metab. Rev. 13(4) 695-714 (1982).
6. A.E. SABOURAUD, M. URTIZBEREA, K. BENMOUSSA, N.J. CANO, and J-M.G. SCHERRMANN, J. Pharm. Pharmacol. 44 1015-1019 (1992).
7. A. SABOURAUD, M. REDUREAU, P. GIRES, M. MARTINET, and J.-M. SCHERRMANN, Drug Metab. Dispos. 21 997-1002 (1993).
8. S.S. MATSUYAMA, L.D. BRAUN, W.H. OLDENDORF, T-K. FU, and L.F. JARVIK, Age 12 107-108 (1989).
9. A. HUNTER and C. KLAASSEN, J. Pharmacol. Exp. Ther. 192 605-617 (1975).
10. R. ROBERTS, C. KLAASSEN, and G. PLAA, Proc. Soc. Exp. Biol. Med. 125 313-316 (1967).
11. J. BLANCHARD, L.M. TANG, and M.E. EARLE, J. Pharm. Sci. 79(5) 411-414 (1990).
12. K. BENMOUSSA, A. SABOURAUD, J.M. SCHERRMANN, D. BROSSARD, and J.M. BOURRE, J. Pharm. Pharmacol. 45 692-696 (1993).
13. K. BENMOUSSA, A. SABOURAUD, J.-M. SCHERRMANN, and J.-M. BOURRE, Pharm. Res. 11 1458-1461 (1994).
14. C. JUSTE, Y. DEMARRE, and T. CORRING, J. Nutr. 113 1691-1701(1983).
15. S. MIURA, H. ASAKURA, M. MIYAIRI, T. MORISHITA, H. NAGATA, and M. TSUCHIYA, Digestion 23 224-231 (1982).
16. M. PAVELKA and A. GANGL, Gastroenterology 84 544-555 (1983).
17. S.G. BARNWELL, P.J. LOWE, and R. COLEMAN, Biochem. J. 220 713-731 (1984).
18. S.G. BARNWELL, I.M. YOUSEF, and B. TUCHWEBER, Biochem. J. 236 345-350 (1986).

19. C.A. BERNHARDT, Food technol. Int. Europe 176-178 (1988).
20. D.J. HAMM, J. Food Sci. 49 419-428 (1984).
21. F.H. MATTSON and R.A. VOLPENHEIN, J. Lipid Res. 13 325-328 (1972).
22. R.A. VOLPENHEIN, D.R. WEBB, and R.J. JANDACEK, J. Toxicol. Environ. Health 6 679-683 (1980).
23. K.J. PALIN, C.G. WILSON, S.S DAVIS, and A.J. PHILLIPS, J. Pharm. Pharmacol. 34 707-710 (1982).
24. D.J.A. JENKINS, A.R. LEEDS,B. SLAVIN, J. MANN, and E.M. JEPSON, Am. J. Clin. Nutr. 32 16-18 (1979).
25. B. SOMMERKAMP and M. HESSER, Food Technol. 44 92-97 (1990).