Interaction of mixed micelles formed from glycocholic acid and lecithin with the protein binding of various drugs

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1 Mixed micelles (MM) formed from glycocholic acid and lecithin are suited to solubilize lipophilic drugs for intravenous use. To test for possible drug–drug interactions, the protein binding of a series of agents known to bind to different sites on albumin (diazepam, warfarin, ketoprofen, frusemide, probenecid) and additionally (prazosin, quinidine, propranolol) or exclusively (disopyramide) to α_1 -acid glycoprotein or to transcortin (prednisolone) was determined in the presence and absence of MM.

2 Concentrations of MM, corresponding to the maximum possible plasma concentration achieved by injecting the highest clinical doses of MM into the systemic circulation, had little or no effect on the unbound fractions of drugs known to bind exclusively to albumin. Only at five times higher MM concentrations were the free fractions substantially increased (by up to 45%).

3 Unbound fractions of drugs bound with high affinity but low capacity to α_1 -acid glycoprotein were increased between 50–85% even at ‘therapeutic’ doses of MM.

4 The present study suggests that drugs solubilized by MM should be given by slow injection or infusion to patients already receiving drugs which are highly bound to α_1 -acid glycoprotein.

Keywords mixed micelles glycocholic acid protein binding interaction albumin α_1 -acid glycoprotein

Introduction

Mixed micelles have been used successfully to solubilize lipophilic drugs thereby allowing their incorporation into intravenous formulations (Steffen & Schmidt, 1978, 1979). Recently, a new diazepam preparation for parenteral use was developed using this principle (Valium-MM®, F. Hoffmann-La Roche & Co. Ltd, Basel, Switzerland). In this preparation mixed micelles are formed from glycocholic acid and lecithin. The components of mixed micelles are

both bound to serum constituents, the bile acid to albumin (Kakis *et al.*, 1977) and lecithin to lipoproteins (Jonas, 1979; Scherphof & Morselt, 1984). The molar amount of glycocholic acid (1.14 mmol) injected in the mixed micelles of the Valium-MM®-type, at the highest intravenous bolus dose of diazepam presently envisaged (6 ml MM, corresponding to 2 μ l MM ml⁻¹ serum), may result in a concentration close to that of albumin in serum (0.677 mM). An interaction

with the binding of drugs, concomitantly present in blood, with a high affinity for this serum protein may therefore occur. Because unbound rather than total drug concentration in blood is generally believed to determine the intensity of pharmacologic effects, protein binding interactions are of clinical interest and have received much attention (for recent reviews see McElnay & D'Arcy, 1983 and Reidenberg & Drayer, 1984). Accordingly, the unbound fractions of model compounds and representative drugs known to have a high affinity for serum albumin (diazepam, warfarin, ketoprofen, frusemide, probenecid) and/or other binding proteins (α_1 -acid glycoprotein: prazosin, propranolol, quinidine, disopyramide; transcortin: prednisolone) were determined in the absence and presence, respectively, of two concentrations of placebo mixed micelles of the Valium-MM®-type formulation.

Methods

Drugs investigated

Depending on the methodology employed to determine the degree of protein binding, labelled or unlabelled drug was used.

Diazepam was labelled with ^{14}C in position 5 of the molecule. The specific activity was $196\ \mu\text{Ci mg}^{-1}$ and the radiochemical purity was better than 99% as determined by thin-layer chromatography (precoated silica gel plates, 0.25 mm, Si-60 F₂₅₄, Merck, Darmstadt, FRG; system: chloroform-acetone 9:1, v:v) and subsequent radioactivity scanning.

[^{14}C]-Warfarin (3- α -acetyl [α - ^{14}C] benzyl-4-hydroxycoumarin) was obtained from Amersham International (Amersham, England). It had a specific activity of $177\ \mu\text{Ci mg}^{-1}$ and a radiochemical purity equal to or better than 98%, as determined by paper chromatography (mobile phase: isopropanol-water 8:2) and by thin-layer chromatography on silica gel in several mobile phases (toluene-methanol 9:1; toluene-dioxan 9:1; toluene-ethyl formate-formic acid 5:4:1) with subsequent radioactivity scanning.

Ketoprofen was either used unlabelled (gift from Ives Laboratories, New York, NY) or with a general ^3H -label (Service des Molécules Marquées, Gif-sur-Yvette, France) of $23\ \text{Ci mmol}^{-1}$ specific activity and > 98% purity. In the buffer phase after dialysis > 96% of the radioactivity represented unchanged drug as assessed by h.p.l.c. (column: Ultrasphere Octyl 5 μ , 4.6 mm i.d., 15 cm length, Altex, Berkeley,

CA; mobile phase: 45% acetonitrile and 0.4% acetic acid in water, pH 4.5; flow rate: $1\ \text{ml min}^{-1}$; detection u.v. 262 nm).

Radioactive prazosin (general ^3H -label; $1.3\ \text{mCi mmol}^{-1}$) was a gift from Pfizer Laboratories Div. (New York, NY). Its purity (> 96%) was determined using an h.p.l.c. method with fluorescence detection (column: Alltech C-8, 4.6 mm i.d., 25 cm length; mobile phase: 39% acetonitrile, 0.15% 5 M sodium hydroxide, 0.15% 85% phosphoric acid in water, pH 2.70; flow rate: $1.5\ \text{ml min}^{-1}$; excitation 254 nm, emission 370 nm cutoff filter).

Prednisolone (Amersham International, Amersham, England) was tritiated at the 2, 4, 6 and 7 positions and had a specific activity of $157\ \text{mCi mg}^{-1}$. Its radiochemical purity was greater than 97.5% as determined by h.p.l.c. on micro-particulate Hypersil silica, using a gradient from chloroform-ethanol-water 469:30:1 to ethanol-water 98:2.

Frusemide (gift from Hoechst, Frankfurt, Germany), probenecid (gift from Astra, Södertälje, Sweden), propranolol (gift from ICI, Macclesfield, England) and disopyramide (Sigma, St Louis, MO) were used as obtained. Quinidine (Siegfried, Zofingen, Switzerland) was purified according to Thron & Dirscherl (1935) to obtain dihydroquinidine-free material.

Mixed micelles

The mixed micelles used (placebo of mixed micelles of the Valium-MM-type, F. Hoffmann-La Roche & Co. Ltd, Basel, Switzerland; Ro 17-7465/001 batch G FER 10004) contained 88.5 mg glycocholic acid and 169.3 mg lecithin per ml aqueous solution. The micellar size in the preparation was in the 100-Å region.

The mixed micelles were stored at 4° C and ampoules were freshly opened just before use.

Determination of protein binding and analytical methods

For each drug studied, blood was collected from healthy volunteers and allowed to clot (30 min). After centrifugation the serum layer was collected, pooled and stored frozen until used. In the pooled sera total protein was in the range of 72–74 g l⁻¹, albumin 45–49 g l⁻¹ and α_1 -acid glycoprotein 0.688–0.700 g l⁻¹.

Two different drug concentrations were used, one at the upper and the other at the lower edge of the therapeutic range (Table 1). At both concentrations, serum aliquots (1 ml) were then combined with 10 μl , 2.0 μl or 0.0 μl of the mixed micelle preparation. In each of the resulting six

Table 1 Unbound drug fractions in the presence and absence, respectively, of mixed micelles

Drug	Serum drug concentration post-dialysis ($\mu\text{g ml}^{-1}$)	Unbound drug fraction with added MM ¹		
		0 $\mu\text{l ml}^{-1}$	2 $\mu\text{l ml}^{-1}$	10 $\mu\text{l ml}^{-1}$
Diazepam	0.4	0.00708	0.00736	0.00832
	1.6	0.00706	0.00736	0.00842
Warfarin	1	0.00693	0.00690	0.00894
	4	0.00687	0.00707	0.00938
Ketoprofen	0.05	0.0075	0.0079	0.0096
	5	0.0076	0.0081	0.0094
Frusemide	2	0.022	0.022	0.029
	10	0.021	0.025	0.029
Probenecid	20	0.076	0.080	0.116
	101	0.178	0.195	0.165
Prazosin	0.0025	0.0279	0.0492	0.124
	0.05	0.0258	0.0477	0.134
Quinidine	0.2	0.0907	0.140	0.251
	2	0.0935	0.142	0.261
Propranolol	0.05	0.089	0.136	0.192
	0.2	0.082	0.148	0.198
Disopyramide	0.57	0.090	0.151	0.336
	4.3	0.355	0.405	0.562
Prednisolone	Tracer (3 ng ml ⁻¹)	0.121	0.125	0.149
	0.1	0.152	0.164	0.180
	1.0	0.294	0.308	0.309

1 Mean of quadruplicate determinations.

drug-containing samples the fraction of drug unbound was determined in quadruplicate.

Equilibrium dialysis was used to determine the fraction unbound of all the drugs investigated with one exception, warfarin, the binding of which was determined by ultracentrifugation at 37° C. The ratio of drug concentrations in buffer and serum after dialysis was taken as an estimate of the unbound (free) fraction (f_u) of drug. Because the volume shift during dialysis was negligible (< 10%) the results were used directly without applying a correction factor.

The equilibrium dialysis was carried out at 37° C with 0.7–1.2 ml aliquots of drug-containing serum against an equal volume of 0.13 M phosphate buffer pH 7.40 $\pm$ 0.02. The time to reach equilibrium for each drug was determined in preliminary studies and ranged from 5 to 8 h.

The radioactivity of ketoprofen, diazepam and prednisolone in the buffer and serum sides was determined by scintillation counting using standard methods. Concentrations of disopyramide, quinidine, frusemide, probenecid and prednisolone in plasma and buffer samples from

dialysis were determined according to previously reported methods (Huang & Øie, 1982; Guentert & Øie, 1980; Hammarlund, 1984; Hartvig *et al.*, 1982; Frey *et al.*, 1980).

Because serum contained an interfering peak with prazosin on h.p.l.c. and the buffer side showed a secondary radioactive contaminant that was not bound to plasma proteins, the following method was used to determine the concentration of prazosin in buffer and serum. Aliquots (200 μl) of sample were mixed with 200 μl methanol containing 250 ng ml⁻¹ cold prazosin, vortexed and centrifuged. The supernatant was collected and 200 μl was injected onto a 10 μC -8 h.p.l.c. column (25 cm $\times$ 4.6 mm i.d., Alltech, Deerfield, Ill.) attached to a commercial h.p.l.c. system (pump: Model 110A, Beckman Instruments, Inc. Berkeley, CA; injector: WISP Model 710B, Waters Assoc. Inc. Milford, MA). The eluent (24% acetonitrile, 0.15% phosphate buffer pH 2.8 in water; flow rate 1.5 ml min⁻¹) was monitored using a fluorescence detector (model FS 970 Schoeffel Instrument Corp., Westwood, NJ; excitation at 245 nm and emission at

370 nm, cutoff filter) and was collected in 0.5 min fractions. Prazosin-containing fractions (6–8 min fractions) were added to 3.25 ml Aquasol® and the radioactivity determined.

Propranolol was determined in serum or buffer samples by h.p.l.c. A 1 ml sample was combined with 0.2 ml NaOH (5 M for serum, 2 M for buffer), aqueous internal standard solution of quinidine $14 \mu\text{g ml}^{-1}$ (100 μl for serum, 50 μl for buffer) and 0.5 ml H_2O . Mixtures were shaken for 10 min with amyl alcohol 1.5% in heptane ($2 \times 5.5 \text{ ml}$) for serum or chloroform, isopropanol 8:2 (10 ml) for buffer and centrifuged at 200–800 g. The organic phase was removed (9 ml) and transferred into a tapered tube and evaporated to dryness under a stream of nitrogen. The residue was taken up in 125 μl mobile phase, sonicated for 2–5 min and a 100 μl aliquot was then injected onto a C-18 $\mu\text{BondaPak}$ 10 μ column of 30 cm length and 3.9 mm i.d. (Waters Assoc. Inc., Milford, MA) protected by a pre-column (4 cm $\times$ 3.9 mm i.d.; Vydac RP-20, 30–44 μ , Macherey–Nagel, Düren, FRG). The mobile phase (flow rate 1.3 ml min^{-1}) was a mixture of methanol–aqueous 0.05 M KH_2PO_4 pH 3.4 45:55 (v:v). Propranolol was determined fluorometrically (model FS 970, Schoeffel Instrument Corp., Westwood, NJ) using an excitation wavelength of 295 nm and a 340 nm emission cutoff filter. The assay showed an average intra-day coefficient of variation (CV) estimated on three occasions of 4.5% at the two concentrations measured; the CV for inter-day variability was less than 6%.

The protein binding of warfarin was determined by ultracentrifugation (MSE Superspeed 65, Crawley, England). A 400 μl aliquot of serum containing the drug was in this case placed into a microfuge tube (400 μl ; Beckman, High Wycombe, England) and centrifuged at 145,000 g for 16 h at 37° C. A 80 μl aliquot of the supernatant was then carefully taken 5 mm below the level of the lipoproteins, placed into the scintillation cocktail (Lumagel, Lumax BV, Holland) and counted (model 1216 Rack beta, LKB Instruments Ltd, S. Croyden, England) for 10 min. In preliminary experiments it was established that the radioactivity was independent of the depth at which the aliquot was taken. The pH of the serum remained at 7.4 for the duration of the experiment. The unbound fraction of warfarin in the sample was calculated from the ratio of radioactivity per unit volume in the supernatant to that in the original serum. The counts in the original serum were taken as the mean of the counts in three 50 μl aliquots.

Statistical analysis

The effect of mixed micelles on the unbound

fraction of a drug in serum was evaluated statistically at every drug concentration by a one-way analysis of variance (ANOVA). A two-way ANOVA with the factors mixed-micelle concentration and drug concentration and an interaction term between these factors was then performed. A statistical significance in the interaction term at $P < 0.05$ was indicative of a difference in the influence of mixed micelles on the drug's binding at the two concentrations.

All ANOVAs and subsequent multiple comparisons between groups by the general linear models procedure were performed using the SAS package (SAS Institute, Gary, NC).

Results

Experimental techniques

The analytical methods used to quantify drug in buffer and serum obtained after dialysis were checked for interference with added mixed micelles. No such interference was detected in any procedure.

The overall reproducibility of assessment of the unbound fraction was reflected in the CV of the quadruplicate determinations made. Very little variability in the determinations was observed. With no drug tested did the average CV in samples with or without added mixed micelles exceed 10%, at either high or low drug concentration tested. This small variability assured a high discriminatory power in the detection of changes in drug binding after addition of mixed micelles to drug-containing serum. To avoid artifacts due to interday variation all binding experiments for a particular drug were made in parallel on the same day.

The methods involving tritiated compounds (ketoprofen, prednisolone) were controlled to exclude artifacts due to a loss of the label during dialysis and/or to a radioactive contaminant not bound to plasma proteins. Buffer collected after dialysis was chromatographed using a specific method and the fraction of total radioactivity associated with unchanged compound was determined. All the tritiated compounds were stable under the conditions of dialysis.

Approximately 10% of warfarin, in a $1 \mu\text{g ml}^{-1}$ protein-free solution appeared to be adsorbed onto the microfuge polyethylene tube during centrifugation and this adsorption would only marginally influence the estimate of the binding of warfarin. This adsorption was unaffected by the presence of a high concentration ($10 \mu\text{l ml}^{-1}$) of the mixed micelle solution.

Binding interactions

Unbound fractions of the various drugs investigated in the presence and absence of mixed micelles (MM) are compiled in Table 1. MM added in a $2 \mu\text{l}$ volume ml^{-1} of serum, corresponding to maximum clinically applied bolus doses of the MM preparation, had little effect on the unbound fractions of drugs known to bind exclusively to albumin (diazepam, warfarin, ketoprofen, frusemide, probenecid; Figure 1). A clear and statistically significant increase of up to 45% was seen, however, in the free fraction of these drugs in presence of the higher concentration of MM, corresponding to at least five times that expected after injecting a 6 ml dose as a rapid intravenous bolus (Table 1). With the exception of probenecid, the effect of MM was similar at both drug concentrations tested, as indicated by an insignificant interaction term in the ANOVA. The binding of probenecid to albumin is saturable in the therapeutic concentration range and a different effect of a displacer at high and at low probenecid concentrations is therefore expected.

In contrast to the drugs bound exclusively to albumin, the binding of drugs interacting not only with albumin but additionally (prazosin, quinidine, propranolol, prednisolone) or exclusively (disopyramide) with other serum proteins was significantly affected at the lower concentration of MM. This was true for the basic drugs binding to α_1 -acid glycoprotein and for prednisolone binding to transcortin, in addition to albumin. A 50–80% increase in the unbound fractions in the presence of $2 \mu\text{l ml}^{-1}$ MM was seen with compounds bound to α_1 -acid glycoprotein (Table 1, Figure 1). At the higher MM concentration ($10 \mu\text{l ml}^{-1}$) the increase was even more marked with free fractions two to three times above baseline values. As expected, the increase was smaller with disopyramide at drug concentrations saturating the high affinity binding site. In the other cases, with linear binding conditions over the concentration range investigated, the displacing effect was of similar magnitude at both concentrations.

Although there was a statistically significant difference in the binding of prednisolone at tracer (3 ng ml^{-1}) and low (100 ng ml^{-1}) serum concentrations in the presence of 'therapeutic' amounts of MM, the increase was small ($<8\%$; Table 1). Larger increases were again seen with the higher amounts of MM added.

Discussion

Testing about 140 drugs bound to albumin,

Figure 1 Influence of mixed micelles on unbound fractions of diazepam (○, $0.4 \mu\text{g ml}^{-1}$), warfarin (□, $1 \mu\text{g ml}^{-1}$) and disopyramide (Δ, $0.57 \mu\text{g ml}^{-1}$).

Sjöholm *et al.* (1979) showed that almost all binding interactions occurred at either the diazepam or warfarin binding site on albumin. The two compounds were therefore selected for the present investigation on binding interactions with MM because they obviously represent markers for the two most important independent binding sites on the albumin molecule (Sjöholm *et al.*, 1979; Sudlow *et al.*, 1976). With both compounds the results found were similar. In absence of MM the free drug fractions corresponded to previously reported values (Guentert, 1984;

Yacobi *et al.*, 1977). In the presence of MM in amounts corresponding to therapeutically introduced doses ($2 \mu\text{l ml}^{-1}$ serum) there were minor (less than 5%) or no differences seen in the unbound drug fraction when compared with control values. At five times higher amounts ($10 \mu\text{l ml}^{-1}$ serum) a more marked increase in the unbound drug fraction occurred. With diazepam the values increased by 18% over control, with warfarin by 37%. As indicated by the insignificant interaction term in the ANOVA the same displacement interaction pattern occurred at both drug concentrations investigated.

Since the MM preparation in the therapeutic dose range did not displace diazepam and warfarin to any significant degree, the absence of an interaction with a series of drugs displacing the two model compounds (Sjöholm *et al.*, 1979) can be assumed. No significant interaction of MM would thus be expected with the serum albumin binding of other benzodiazepines, of non-steroidal anti-inflammatory drugs such as indomethacin or those derived from propionic acid (e.g. flurbiprofen, ibuprofen, ketoprofen, naproxen) and of antidiabetic drugs of the sulfonylurea type (e.g. glibenclamide, tolbutamide), all drugs bound strongly to the diazepam site on albumin. Furthermore, based on our observations with warfarin no interaction is expected with strongly bound sulphonamides (e.g. sulphadimethoxine, sulphamethizole, sulphafurazole, sulphamoxazole) and penicillin derivatives (e.g. cloxacillin, dicloxacillin, flucloxacillin), analgesics like oxyphenbutazone, diflunisal, salicylic acid derivatives, or commonly used diuretics (e.g. frusemide, chlorothiazide, bumetanide). The results obtained with ketoprofen, probenecid and frusemide agree with these extrapolations. Because of the relatively low increase in the unbound fraction of drugs bound to albumin at clinically relevant concentrations of MM, no clinical consequences are expected for such drugs when MM are administered.

The effect of MM on prednisolone both at tracer doses and at higher concentration was small, similar to drugs exclusively bound to albumin. As prednisolone is almost entirely bound to transcortin at tracer concentrations (3 ng ml^{-1}) and mainly to albumin at higher levels ($1 \mu\text{g ml}^{-1}$) these results indicate that MM has little effect on the binding to both transcortin and albumin.

While albumin is essentially the only plasma protein that binds acidic drugs, basic compounds bind additionally or exclusively to other plasma constituents. Tricyclic antidepressants, such as imipramine (Borgå *et al.*, 1977; Javai *et al.*, 1983) and amitriptyline (Tinguely *et al.*, 1985),

the phenothiazine chlorpromazine (Piafsky *et al.*, 1978), the β -adrenoceptor blockers alprenolol (Borgå *et al.*, 1977) and propranolol (Sager *et al.*, 1979), antiarrhythmics such as lignocaine (Piafsky & Knopfert, 1978) and quinidine (Nilsen & Jacobsen, 1975) are all strongly bound to α_1 -acid glycoprotein (AAG). In many cases the extent of binding correlates well with the total concentration of this acute phase reactant protein.

Since no significant changes in binding of drugs with affinity to albumin alone were observed in presence of $2 \mu\text{l ml}^{-1}$ MM it was quite unexpected to find larger increases in unbound fractions of drugs bound additionally to other serum proteins. Binding of the basic drugs investigated (prazosin, quinidine and propranolol) was significantly decreased when dialyzed in the presence of MM amounts corresponding to a 6 ml volume injected into man. The fractions unbound increased between 50–80% and this extent was similar at both drug concentrations investigated. With higher amounts of the MM preparation added the displacement was even more pronounced. The fact that free fractions of disopyramide, a drug bound almost exclusively to AAG (Norris *et al.*, 1984), were also increased markedly, suggests that the binding interaction is occurring with AAG binding sites, rather than with those on albumin. The reason for the lack of a marked difference in the extent of an interaction with the basic drugs investigated is not clear since the contribution of AAG to overall serum binding is, according to the literature, different with the drugs chosen. Prazosin is bound to an equal degree in plasma to both albumin and AAG (Rubin & Blaschke, 1980). With no effect on albumin binding, a somewhat lesser effect of the MM solution on prazosin would be expected than seen with drugs solely (disopyramide (Lima & Salzer, 1981)) or mainly (propranolol) (Alvan *et al.*, 1983)) bound to AAG. However, the displacement of prazosin was similar to that seen for disopyramide at non-saturable conditions and not in between the values for disopyramide and drugs exclusively bound to albumin. The 'buffering' effect of albumin, which is expected when a drug is bound equally to albumin and AAG, seems to be negligible. An explanation involving two different binding sites on AAG is unlikely since only one common binding site to AAG for basic drugs has been identified so far (Müller & Stillbauer, 1983; Goolkasian *et al.*, 1983). Much of the literature on the relative contribution of a protein to overall binding is derived from experiments using purified proteins, but such results may not reflect the situation in whole serum adequately (Pike *et al.*, 1982, 1984). Certainly,

the binding of drugs to AAG can be increased in the presence of albumin (Owens *et al.*, 1983). It is therefore conceivable that this interaction is modified in the presence of a ligand binding to albumin.

The conclusions from our study on an interaction of MM constituents with a high affinity but low capacity binder are well supported by observations with disopyramide. With this drug the binding site becomes saturated at therapeutic concentrations (Hinderling *et al.*, 1974). Because at higher concentrations a larger drug fraction is unbound, the displacing effect of MM would be expected to be less pronounced at the higher drug concentration chosen, which was indeed the case.

How drugs are displaced from binding sites on AAG by the mixed-micelle preparation is not clear at present. In principle both components of the MM could be responsible for the interaction. Lecithin binds to lipoproteins (Jonas, 1979; Scherphof & Morselt, 1984) as do several drugs and vitamins (Hobbelen *et al.*, 1975; Takahashi *et al.*, 1977; Lemaire & Tillement, 1982), in particular those with basic properties (Bickel, 1975; Chen & Danon, 1979; Pike *et al.*, 1982). However, for several reasons, lecithin is an unlikely candidate to cause the observed interaction. The amount of lecithin introduced into the plasma by intravenously injecting 6 ml MM of the diazepam type corresponds to approximately 30% of endogenously present phosphatidylcholine (Wissenschaftliche Tabellen Geigy, 1979). However, even when the MM are broken down in plasma, very little lecithin is present in the monomeric state. Bilayer fragments combine with the high density lipoprotein fraction and the effective lecithin concentration is therefore reduced. The lack of saturation of drug binding to lipoproteins points to a partitioning of drug into the lipoprotein fraction rather than to the involvement of specific binding sites. Furthermore, for several of the basic drugs displaced by MM in our study (quinidine, propranolol, disopyramide) lipoproteins do not contribute to their overall binding in plasma (Pike *et al.*, 1984; Edwards *et al.*, 1984). Glycocholic acid is much more likely to be responsible for the interaction. Conjugated bile acids are highly bound to albumin with free fractions for glycocholic acid around 15% (Kakis *et al.*, 1977; Cowen *et al.*, 1975). Initial concentrations reached in serum after a 6 ml MM bolus

injection are approximately 0.38 mM, close to that of endogenous serum albumin (0.68 mM). Although an interaction is theoretically possible, it is not clear by what mechanism(s) drugs bound partially or almost entirely to AAG could be displaced by an acidic compound reportedly bound to albumin. However, as AAG binding of drugs may increase in the presence of albumin (Owens *et al.*, 1983), conceivably the effect is indirect. The presence of MM might change the interaction between the two proteins with a resulting decrease in overall binding of drug. That the results are an artifact caused by entire micelles crossing the semipermeable membrane during dialysis and trapping basic drugs in the buffer compartment, can be excluded. Mixed micelles cannot cross the dialysis membrane as was indicated by the absence of detectable lecithin in the buffer compartment after dialysis.

The clinical relevance of the displacement of drugs bound to AAG by MM is uncertain but may be potentially significant. An increase in the unbound fraction of drugs will cause a significant increase of the unbound (active) concentration in plasma and subsequently extravascularly as the drug diffuses out of the vascular space. The drug's activity and/or side effects may thereby be increased, but fleetingly. *In vivo* distribution and elimination processes rapidly reduce the serum concentrations of glycocholic acid injected. Distribution and elimination half-lives of around 2 and 10 min, respectively, have been observed for this bile acid in man after intravenous bolus injection (Cowen *et al.*, 1975; Gilmore & Thompson, 1978). Dose-independence, i.e. linearity of the elimination kinetics, was established over a large dosage range (70–525 mg) (Korman *et al.*, 1975) including the doses to be applied in form of MM. Even after the highest investigated dose of 7.5 mg kg⁻¹, glycocholic acid peripheral predose concentrations below 1 µM were achieved again within approximately 10 min. Moreover, the high hepatic extraction ratio for glycocholic acid (0.85; Gilmore & Thompson, 1978) assures rapid removal of this constituent. Nevertheless, it appears advisable to inject drug preparations solubilized with MM slowly, thereby lowering the concentrations of MM constituents present at any time in plasma and hence reducing the potential for an interaction with other drugs present in blood and highly bound to plasma proteins.

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(Received 16 January 1986,
accepted 7 January 1987)