Studies on the Excretion of Diazepam and Nordazepam into Milk for the Prediction of Milk-to-Plasma Drug Concentration Ratios

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The influence of varying protein and fat content in milk of New Zealand White rabbits on the milk-to-plasma drug concentration (M/P) ratio of diazepam and its metabolite nordazepam following administration of diazepam was studied. At various time points after littering, a bolus dose (1.5 mg/kg) followed by a 26-hr infusion (1.8 mg/h) of diazepam was administered to freely moving rabbits via a jugular vein catheter. Milk and blood samples were collected to allow characterization of milk composition and quantitative determination of diazepam and nordazepam in milk and plasma. At steady state diazepam showed M/P ratios between 3.7 and 9.5, whereas nordazepam showed ratios between 2.1 and 4.3, respectively. The relative importance of milk protein binding and milk-fat partitioning for the excretion of a drug into milk depended on the drug's affinity to milk fat. A stepwise multiple regression analysis suggested that observed M/P ratios of diazepam could be explained by considering the fat content of milk alone. Nordazepam with a lower solubility in milk fat showed M/P ratios which could be best explained by considering protein and fat concentrations together. Using the data from the infusion studies, two recently published diffusional models to predict M/P ratios were evaluated. Neither model could accurately predict the M/P ratios of diazepam and nordazepam observed in rabbits. However, after extending the model described by Atkinson and Begg to take the actually measured partitioning between skim milk and milk fat into account, a great improvement in the predictive power for observed M/P ratios occurred. Therefore, to estimate the potential for a drug to accumulate in milk using the developed relationship, the following parameters should be measured: the creamatocrit, the skim-to-whole milk drug concentration ratio, and the free, nonionized fractions of a drug in plasma and in milk.

KEY WORDS: diazepam; nordazepam; benzodiazepines; milk; milk composition; milk-to plasma drug concentration ratio; protein binding; infusion.

INTRODUCTION

Passive diffusion is the most common mechanism by which drugs pass from the bloodstream into milk (1). Only unbound, nonionized drug can cross biological membranes, and its concentration difference across the lipoid barrier regulates the amount of drug excreted into milk. Therefore, the binding of drugs to plasma and milk proteins and solubility in milk fat influence the active concentration gradient and determine the amount of drug trapped in milk and hence the resulting milk-to-plasma drug concentration (M/P) ratio (2).

Recently, Fleishaker *et al.* (3) [Eq. (1)] and Atkinson and Begg (4) [Eq. (2)] published relationships to predict the M/P ratio of a drug once steady-state conditions are achieved. The proposed models are based on the partitioning of nonionized, unbound drug between skim milk and milk fat:

$$\mathrm{M/P} = \frac{fu_p * f_p}{fu_m * f_m * (\mathrm{S/M})} \quad (1)$$

$$\mathrm{M/P} = \frac{fu_p * f_p}{f_m}\left[\frac{(1 - \mathrm{cr})}{fu_m} + \mathrm{cr} * P_m\right] \quad (2)$$

where fu_p and fu_m are the fractions of drug unbound in plasma and milk, respectively, f_p and f_m are the fractions of drug nonionized in plasma/milk, (S/M) is the skim-to-whole milk drug concentration ratio, cr is the creamatocrit (measure for fat concentration in milk; see below), and P_m is the milk lipid/milk ultrafiltrate partition coefficient, which can be calculated from the oil/water partition coefficient (O/W) (4,5):

$$\log P_m = -0.88 + 1.29 * \log (\mathrm{O/W}) \quad (3)$$

Neither of the two equations [Eqs (1) and (2)] has been extensively checked *in vivo*, and it is not known whether they can be generalized and give reliable information about the M/P ratio of drugs with different chemical structures.

The present study was undertaken to determine the influence of varying total protein and fat content in milk of New Zealand White (NZW) rabbits on the M/P ratios of diazepam and its major metabolite nordazepam (*N*-desmethyldiazepam) under steady-state conditions. Such conditions were achieved during a 26-hr infusion of diazepam through an implanted jugular vein catheter to freely moving rabbits. Additionally, the two diffusional models to predict M/P ratios [Eqs. (1) and (2)] were evaluated by comparing model predictions with M/P ratios observed *in vivo*.

MATERIALS AND METHODS

Model Compounds

Diazepam infusion solution consisted of a sterile mixture of 25.0 ml Valium MM (10 mg/2 ml; Batch B0138, F. Hoffmann-La Roche Ltd., Switzerland) and 25.0 ml 0.9% sodium chloride solution (Vifor, Switzerland). The resulting diazepam concentration was 2.5 mg/ml and the volume weight was 1.017 g/ml. The physicochemical properties of diazepam and its metabolite nordazepam are listed in Table I.

Experimental Animals

Six pregnant NZW rabbits were obtained from K. Thomae GmbH, Germany. The mean body weight after whelping was 3466 ± 235 g and the mean litter size was six. The animals were housed in steel cages at a room temperature of 22°C and a relative humidity of 55%. They had free access to fodder (Nafag No. 814, Kliba, Switzerland) and water at any time. A 12-hr light/dark cycle was maintained. The day of whelping was considered to be day 1 postpartum

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Table I. Physicochemical Properties of Model Compounds

Parameter	Diazepam	Nordazepam
pK_a[a]	3.30	3.48
O/W[a]	646	676
P_m[b]	556	590
S/M[c]	0.17	0.47
fu_p (%)[c]	7	6
fu_m (%)[c]	10	8

[a] Data taken from Refs. 21 and 22.
[b] Calculated with Eq. (3).
[c] Mean values of this study measured in rabbit plasma (concentration range: diazepam, 350–470 ng/ml; nordazepam, 390–420 ng/ml) and milk (concentration range: diazepam, 1990–3400 ng/ml; nordazepam, 980–1480 ng/ml).

(pp). After whelping the females were separated from their offspring. Every 24 hr (usually at 9 AM) they had access to the litter box for nursing for about 10 min. At the end of the study the animals were killed by administering sodium pentobarbital intravenously.

Implantation of Jugular Vein Catheter

The animals were anesthetized with a solution of sodium pentobarbital (about 40–50 mg/kg). The Silastic end of the catheter (see below) was inserted into the jugular vein up to the heart.

The catheter used was a 10-cm-long Silastic tube (No. 602-175; Dow Corning, USA) connected to a 15-cm-long polyethylene tube (P.A. Stoss, Germany) using a stainless-steel tube of 0.5-cm length, which was produced in F. Hoffmann-La Roche's workshop. The dead volume of the catheter was about 130 μl. The catheter was kept under the skin and was exteriorized behind the neck of the animals. The catheter was filled with 130 μl heparin solution (Liquemin, 250 UI/ml; Roche) and closed with a little stainless-steel plug. Beginning on the second day after surgery the catheter was flushed twice a week with Liquemin (Roche), 100 UI/ml (about 150 μl).

Infusion Pumps

For continuous drug delivery to freely moving rabbits, portable infusion pumps (Travenol Infusor; Travenol Inc., USA) were used. The mean flow rate of the Infusor filled with infusion solution was about 0.72 g/hr, which equaled a delivery rate of 1.8 mg diazepam/hr (R_0). The coefficient of variation (CV%) of the mean flow rate of three tested pumps was 2%.

Drug Administration and Infusion Technique

The infusion pump was filled with 45 ml of infusion solution containing diazepam, 2.5 mg/ml (see Model Compounds). The Infusor was weighed at the beginning and the end of the infusion and the difference between these values divided by the duration of the infusion provided an estimate of the mean flow rate. After collecting baseline blood and milk samples, a loading dose of 1.5 mg diazepam (Valium MM, Roche) per kg body weight was administered through the jugular catheter and the Infusor was then connected to the jugular catheter. Afterward the infusion was started (duration, 26 hr) and the Infusor was fixed on the back of the rabbit with a self-constructed rucksack (6). Blood samples were taken (5, 20, 60 min) and the rabbits were brought back to their cages. Blood and milk samples were taken again after about 2.5 hr. Afterward the rabbits had access to the litter box for nursing for about 10 min with their rucksacks. After 10 hr blood was taken and the rabbits were milked again, but this time they had no access to the litter box. The next morning (26 hr) they were milked, blood was taken, and the Infusor was disconnected from the catheter. Afterward the puppies were nursed. The first infusions were administered between day 7 and day 19 pp, and the procedure was repeated several times in five of the six animals at intervals of about 10 days in each animal until milk production stopped.

Collection of Milk and Blood Samples

The rabbits were milked using a vacuum milking aid (6) until approximately 10 ml of milk was collected. After collection the milk samples were frozen at −70°C within 1 hr for later use in binding and biochemistry determinations.

Blood samples (about 3 ml each) were taken during the infusions from the rabbit's ear vein with a 1.20 × 38-mm needle. The anticoagulant used was 25% potassium oxalate, 1:100. The blood samples were placed on ice and were centrifuged for 15 min at 3000*g* within 1 hr, and the blood plasma was stored at −70°C.

Drug Concentration Measurement

Diazepam and nordazepam in plasma and milk samples were quantitated by a previously reported and characterized HPLC method including UV detection (7). The assay involves extractions with diethyl ether and an acid cleanup step. All major metabolites could be separated. The limit of determination was 20 ng/ml for diazepam and 15 ng/ml for nordazepam.

Characterization of Milk Samples

Immediately after cooling the fresh milk samples to room temperature (22°C), milk pH was measured aerobically with a Metrohm 632 (Mettler, Switzerland). Aliquots of milk samples were defatted by centrifugation for 30 min at 3000*g* for protein concentration and protein binding determinations.

Total milk protein concentration in skim milk was determined with a commercial protein determination kit (No. 609-A; Sigma, USA) making use of a combined biuret/Lowry reaction (8). The mean coefficient of variation of 52 duplicated protein concentration determinations was 4.7%.

Milk-fat concentration was determined as creamatocrit: A milk sample was drawn into a nonheparinized hematocrit tube (Goldseal, USA) and centrifuged at 12,000*g* for 15 min. The creamatocrit was expressed as the percentage of the length of the cream layer to the length of the whole milk column (9). The creamatocrit values given in this study are

the means of six such determinations. The coefficient of variation of the replicate determinations was below 2.5%.

Remaining milk-fat concentration in skim milk was estimated as triglyceride (TG) concentration with a commercial TG determination kit (No. 405-A, Sigma, USA) (10).

The S/M drug concentration ratios were calculated as the ratio between the drug concentration in skim and that in whole milk.

Binding Studies

Protein binding was determined by equilibrium dialysis, using a dialysis membrane with a cutoff of 12,000 daltons (Union Carbide, USA). Before use, the membrane was soaked in distilled water (10 min), in absolute alcohol (15 min), and finally, in buffer (30 min) (11). The buffer used was a 1/15 *M* phosphate buffer adjusted to milk/plasma pH, which was made isocryoscopic with lactose monohydrate (Merck, Germany) for the milk samples and with sodium chloride (Merck, Germany) for the plasma samples according to Pharmacopoea Helvetica VI. Aliquots of 800 µl of skim milk or plasma were dialyzed with 800 µl of the appropriate buffer solution in Plexiglas dialysis cells (F. Hoffmann-La Roche Ltd., Switzerland). The cells were rotated (8 rounds/min) for 5 hr in a Dianorm apparatus (Diachema, Switzerland), with the water bath set at 37°C. Each determination was carried out in duplicate. In all determinations the volume shift was below 11%. Binding of drug to the dialysis membrane and/or dialysis cell was below 6.5%. The alterations in pH before and after dialysis were, at most, 0.23 pH unit. Protein binding of diazepam and nordazepam was independent of concentration within the small concentration range encountered in rabbit skim milk and plasma for binding measurements (see Table I). Free fractions (*fu*) were calculated by dividing the drug concentration measured in the buffer by the drug concentration measured in the protein solution. Fractions bound (*fb*) were calculated by (1 − *fu*).

Data Analysis

All concentration–time data were plotted first for visual inspection. Total-body clearance was calculated for the infusion data as

$$CL = \frac{R_0}{C_{p_{ss}}} \tag{4}$$

where R_0 is the infusion rate (amount of drug/time) and $C_{p_{ss}}$ the steady-state plasma drug concentration level (mean of observed C_p values at time 10 and 25 hr).

The observed milk-to-plasma drug concentration ratio (M/P_{obs}) was calculated as

$$M/P_{obs} = \frac{C_m}{C_{p_{mean}}} \tag{5}$$

where C_m is the measured milk concentration of diazepam or nordazepam at time 2.5, 10, or 25 hr, and $C_{p_{mean}}$ is the average plasma drug concentration in a milk sampling interval (0–2.5, 2.5–10, or 10–25 hr, respectively) obtained by dividing the corresponding AUC (calculated using the linear trapezoidal rule) by the sampling interval.

The nonionized fraction of diazepam or nordazepam in plasma (f_p) and milk (f_m) at a given pH was calculated as

$$f = \frac{1}{1 + 10^{(pK_a - pH)}} \tag{6}$$

The predicted milk-to-plasma drug concentration ratio (M/P_{calc}) was calculated as described in Eqs. (1) and (2).

Equation (2) was extended in the following way to take the actually measured partition between skim milk and fat into account:

$$M/P = \frac{fu_p * f_p}{f_m}\left[\frac{(1 - cr)}{fu_m} * S/M + cr * P_m * (1 - S/M)\right] \tag{7}$$

Correlations of observed M/P ratios with protein and fat contents and correlations between M/P_{obs} and M/P_{calc} were determined by linear and multiple regression analysis using the computer program RS/1 (Bolt, Beranek and Newman, Cambridge, MA) and accepting a significance level α of 0.05. To select the model best describing the observations, Akaike's information criterion (AIC) (12) was used:

$$AIC = N * \ln R + 2 * p \tag{8}$$

where N is the number of experimental data points, R is the residual sum of squares of the calculated and observed values, and p is the number of parameters in the estimated model. The model with the smallest AIC value was considered to describe the experimental data best.

RESULTS

The animals tolerated the jugular vein catheter well and did not show any evidence of infections at the catheter site. The total protein concentrations and the creamatocrit in rabbit milk ranged between 84 and 140 g/L and between 13.6 and 29.2%, respectively. The pH ranged from 6.66 to 7.78. The remaining milk fat in skim milk was less than or equal to 0.2%.

In Fig. 1 typical plasma and milk concentration–time

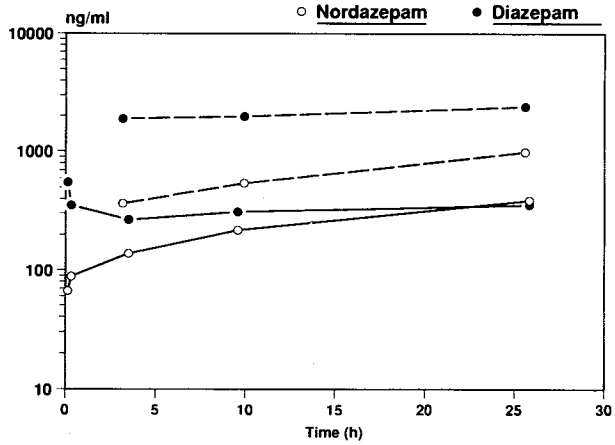

Fig. 1. Typical concentration–time curve of diazepam and nordazepam during infusion of diazepam. Animal Tina, 11 days pp, R_0 = 1.79 mg/hr. (●) Diazepam; (○) nordazepam; (——) plasma; (--) milk.

curves during infusion are shown. The corresponding parameters for one individual animal and the range in all six animals during the first infusion are listed in Table II. Diazepam clearances calculated from steady-state concentrations in all experiments were within 1.16–1.90 L/hr/kg.

Diazepam and nordazepam were both extensively excreted into milk, reaching higher concentrations in milk than in plasma. After about 2.5 hr of infusion, steady-state plasma concentrations of diazepam were achieved. The plasma concentration of nordazepam increased throughout the 26-hr experiment, reflecting the slow elimination of this metabolite. Diazepam concentrations in milk reached a steady state between 2.5 and 10 hr after the start of the infusion, while nordazepam seemed to increase slightly over the entire observation period. Nordazepam was bound to a higher degree in skim milk and plasma than diazepam, but both drugs were less bound in milk than in plasma (see Table II). Diazepam distributed more extensively into milk fat than nordazepam, resulting in a much lower S/M ratio (diazepam, about 0.17; nordazepam, about 0.47). Diazepam, with its high solubility in milk fat (13), showed higher M/P ratios than the less milk fat-soluble nordazepam. The M/P ratios for diazepam and nordazepam correlated significantly with either the total protein or the fat content (Figs. 2 and 3). A multiple regression analysis suggested that the observed M/P ratios for diazepam could be predicted by considering the fat content alone, whereas the observed M/P ratios for nordazepam could be explained best by considering the total protein and fat content together.

M/P ratios calculated with Eq. (1) tended to be lower, whereas M/P ratios calculated with Eq. (2) were several times higher than the actually observed values. When using Eq. (7) the calculated M/P ratios were within narrow proximity of the actually found M/P_{obs} values. Statistically significant correlations between M/P_{calc} and M/P_{obs} (nordazepam, $P = 8.0 * 10^6$, $r = 0.778$) could be found. In contrast to Eqs. (1) and (2), a linear regression analysis between M/P_{calc} based on Eq. (7) and M/P_{obs} including both drugs together even suggested that the obtained single regression line described the data best ($P = 1.4 * 10^{-17}$, $r = 0.906$) (see Fig. 4).

Table II. Parameters Important for Milk Excretion During Diazepam Infusion in Animal TINA (11 days pp, R_0 = 1.79 mg/hr) and Range in Six Animals After First Infusion (in Parentheses)

Parameter	Diazepam	Nordazepam
2.9 hr		
$C_{P_{mean}}$ (ng/ml)	315 (315–414)	108 (68–116)
C_m (ng/ml)	1890 (1270–2781)	365 (190–365)
M/P	6.00 (3.67–6.73)	3.36 (2.38–4.17)
fu_p	0.075 (0.074–0.078)	0.063 (0.061–0.063)
fu_m	0.102 (0.102–0.198)	0.077 (0.077—0.091)
S/M	0.19 (0.15–0.22)	0.50 (0.42–0.55)
10.8 hr		
$C_{P_{mean}}$ (ng/ml)	288 (272–288)	178 (115–178)
C_m (ng/ml)	1970 (1870–2207)	542 (370–570)
M/P	6.84 (6.11–7.89)	3.05 (2.21–3.33)
fu_p	0.069 (0.060–0.077)	0.057 (0.052–0.063)
fu_m	0.100 (0.100–0.114)	0.073 (0.073–0.084)
S/M	0.20 (0.15–0.20)	0.54 (0.42–0.54)
26.6 hr		
$C_{P_{mean}}$ (ng/ml)	332 (282–405)	302 (136–344)
C_m (ng/ml)	2399 (2188–3421)	980 (509–1480)
M/P	7.22 (7.14–8.44)	3.24 (3.10–4.30)
fu_p	0.081 (0.069–0.081)	0.068 (0.059–0.068)
fu_m	0.094 (0.094–0.081)	0.071 (0.071–0.083)
S/M	0.18 (0.16–0.21)	0.049 (0.45–0.55)
CL (L/hr/kg)	1.45 (1.16–1.78)	

DISCUSSION

The steady-state plasma concentration of diazepam was targeted to be about 300 ng/ml, a concentration close to that seen in humans after 5 mg of diazepam three times daily (14). Because of the pK_a values of diazepam and nordazepam, the considerable intraindividual differences in rabbit milk pH did not significantly affect the nonionized fractions of these drugs in milk.

Already 2.5 hr after the start of the infusions steady-state plasma concentrations were achieved. With a half-life of diazepam of about 3 to 4 hr in the rabbit, this rapid achievement of steady state was possible only with the help of the appropriate loading dose. Diazepam clearances calculated from these steady-state plasma concentrations (1.16–

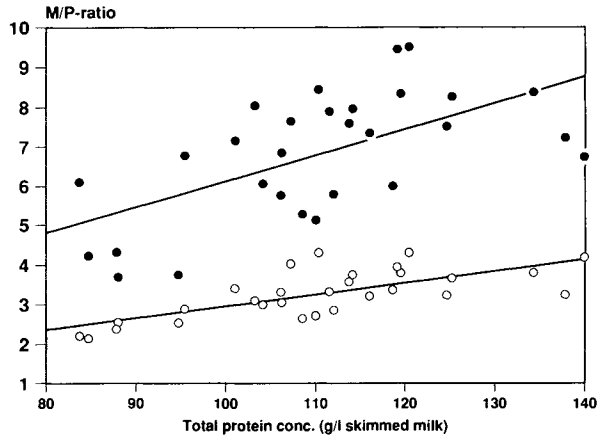

Fig. 2. Relationship between observed M/P ratios and total protein concentration in rabbit skim milk. (●) Diazepam; (○) nordazepam; (——) calculated linear regression line.

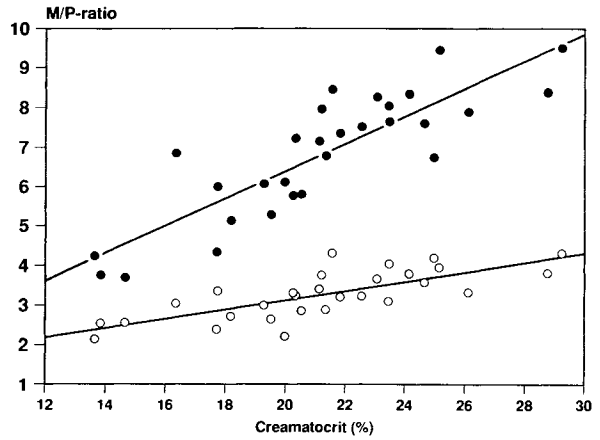

Fig. 3. Relationship between observed M/P ratios and fat concentration in rabbit milk. (●) Diazepam; (○) nordazepam; (——) calculated linear regression line.

1.90 L/hr/kg) were very similar to those found by Fleishaker *et al.* after single-dose experiments (1.30–2.78 L/hr/kg) (3).

The mean S/M ratio found for diazepam in rabbit milk (0.17) was in accordance with the value reported by Fleishaker *et al.* [0.16 (3)] and is similar to that observed in humans (0.19). The S/M ratio for nordazepam in the rabbit (0.47) is quite different from that found in human milk [0.18 (15)]. It is possible that the different solubilities in rabbit and human milk fat might be explained by different milk-fat compositions (16).

Two main factors determining excretion of drugs into milk were considered in the present investigation: milk-fat partitioning and milk protein binding. Fat concentration in rabbit milk influenced the observed M/P ratios, resulting in a statistically significant positive correlation between milk-fat concentration (creamatocrit) and observed M/P ratios. However, the importance of this influence apparently is dependent on the milk-fat solubility of the drug. Thus, the influence was more marked with the highly milk fat-soluble diazepam and to a lower degree with nordazepam. Beside fat content, total milk protein concentration in rabbit milk also influenced the observed M/P ratios, resulting in statistically

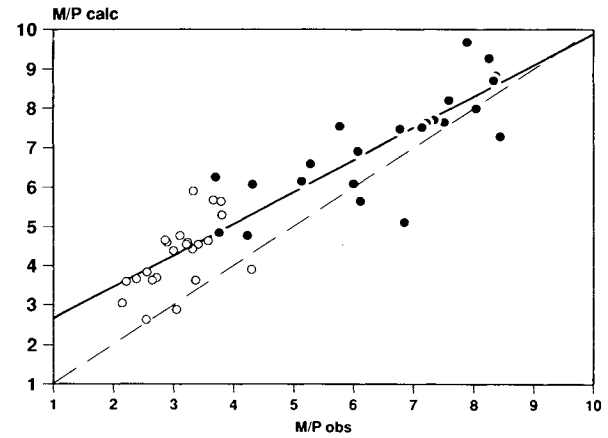

Fig. 4. Relationship between M/P ratios calculated with Eq. (7) and observed M/P ratios. (●) Diazepam; (○) nordazepam; (——) calculated linear regression line through all data points (see text); (---) line of identity.

significant correlations between total protein concentration and observed M/P ratios for diazepam and nordazepam. However, for diazepam the observed M/P ratios in rabbits could be sufficiently well predicted by considering milk-fat content alone, and drug binding to milk proteins hardly affects the observed M/P ratios. In contrast, the observed M/P ratios for nordazepam could be explained satisfactorily only by considering fat and total milk protein concentration together. Thus, the relative influence of milk protein binding depends on the milk-fat solubility of a drug.

These findings may have clinical consequences. Human milk-fat concentration increases during the lactation period from about 29 g/L in colostral milk to 45 g/L in mature milk, and milk may contain more fat at the end of a feeding session than at the beginning (17). Therefore, alterations in human M/P ratios during the lactation period and even over a feeding session due to different milk-fat concentrations may be possible. This presumption is supported by our results in rabbits (Fig. 3) and the results of Matheson and Skjaeraasen 1988 (18) as well as the findings of Matheson *et al.* in 1990 (19), where variations in human milk fat concentrations influenced markedly the M/P ratios of the very lipophilic drugs nortriptyline and flupentixol and of the environmental organochlorine pollutants PCBs and DDE.

In contrast to milk-fat concentration, protein concentration in human milk drops during the first days postpartum. A change from higher M/P ratios in the first days after birth to lower ratios in the later lactation period can therefore be expected for drugs with a low solubility in milk fat. Depending on the drug characteristics, it is also possible that the influences of milk-fat and milk protein concentrations compensate each other, resulting in a constant M/P ratio during the lactation period.

For diazepam and nordazepam no statistically significant correlation between M/P ratios calculated with the help of Eq. (1) and actually observed M/P ratios could be found. Interestingly, Fleishaker *et al.*, proposing this relationship (3), also could not find a correlation between M/P_{calc} and M/P_{obs} for diazepam when using all their observed data points. In contrast, they could adequately predict M/P ratios for other drugs, namely, phenobarbitone, propranolol, and phenytoin. These three drugs show S/M ratios greater than 0.8, indicating a low distribution into milk fat. These results suggest that the relationship proposed by Fleishaker *et al.* to predict M/P ratios is restricted to drugs which are not as highly milk fat-soluble as diazepam or nordazepam. It seems that solubility in milk fat is insufficiently considered in their diffusional model. In contrast to Eq. (1), statistically significant correlations between the M/P ratios calculated with Eq. (2) and the actually observed M/P ratios were found. However, the calculated M/P ratios were too high for nordazepam and diazepam. The reason for this deviation might be the use of Eq. (3) to calculate the milk lipid/milk ultrafiltrate partition coefficient (P_m) used in Eq. (2). In this calculation a linear relationship between P_m and the logarithm of the O/W partition coefficient of a drug is assumed. The calculated P_m is 590 for nordazepam and 556 for diazepam, suggesting similar solubilities of diazepam and nordazepam in milk fat. However, based on the S/M ratio, this is not true. Diazepam accumulates much more in milk fat than nordazepam, resulting in a much lower S/M ratio. This indicates

Table III. Comparison of Three Diffusional Models[a] to Predict M/P Ratios in Humans

	M/P ratio	
	Diazepam	Nordazepam
Observed values (20)	0.13–0.18	0.20–0.35
Predicted values[b]		
Eq. (1)	0.40	0.26
Eq. (2)	0.61	0.65
Eq. (7)	0.48	0.49

[a] Equations (1), (2), and (7) in text.
[b] Human data were taken from Ref. 5 and the creamatocrit was set to 3.4% (equals a mean fat concentration of about 31 g/L).

that P_m does not always parallel the log (O/W) partition coefficient. Greatly improved predictions of the observed M/P ratios result (see Fig. 4) when Eq. (2) is extended to take the actually measured partition between skim milk and milk fat (S/M) ratio into account as is done in Eq. (7). Additionally, when using Eq. (7) to predict observed M/P ratios, a single regression line for diazepam and nordazepam between M/P_{calc} and M/P_{obs} results. When comparing Eq. (2) with Eq. (7), the model selection criterion AIC indicated that the extended diffusional model is better suited to predict M/P ratios than Eq. (2) for both drugs. Furthermore, no limitations concerning the lipophilicity of drugs as seen with Eq. (1) occurred.

Applicability of Eqs. (1), (2), and (7) for M/P ratio predictions across species was compared by applying them to literate data from humans (Table III). All three equations predict higher than observed M/P ratios for diazepam. Equation (2) from Atkinson and Begg shows the highest deviations from real values. But it rightly predicts that M/P ratios for nordazepam can be higher than those for diazepam (20). In contrast, Eq. (1) from Fleishaker *et al.* predicts M/P ratios most closely but fails to predict the actually observed higher M/P ratio of nordazepam compared to diazepam. Additionally, no statistically significant relationship between M/P_{calc} and M/P_{obs} could be found. Suggesting a "worst-case analysis," Eq. (7) should give the most reliable predictions for actually observed M/P ratios. However, only extensive additional research using a variety of conditions (variable milk composition, drugs with varying chemical structures and lipophilicities) can show whether this extended model allows reliable predictions, in general, and to what extent predictions across species and across chemical groups can be made.

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NOMENCLATURE

AIC	Akaike's information criterion
AUC	Area under the drug concentration–time curve
calc	Calculated
CL	Total-body clearance
C_m	Whole milk drug concentration
C_p	Plasma drug concentration
cr	Creamatocrit
CV%	Coefficient of variation
f	Fraction nonionized
fb	Fraction bound
fu	Fraction unbound
m	Milk
MM	Mixed micelles
M/P	Milk-to-plasma drug concentration ratio
MW	Molecular weight
NZW	New Zealand White (rabbit)
obs	Observed
O/W	Octanol/water partition coefficient
P	Plasma
P_m	Milk lipid/milk ultrafiltrate partition coefficient
R_0	Infusion rate
S/M	Skim-to-whole milk drug concentration ratio
TG	Triglycerides

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