

Midazolam and nitrazepam in the maternity ward: milk concentrations and clinical effects

I. MATHESON^{1,2}, P. K. M. LUNDE^{1,2} & J. E. BREDESEN²

¹Department of Pharmacotherapeutics, University of Oslo and ²Section of Clinical Pharmacology and Toxicology, The Central Laboratory, Ullevaal Hospital, Oslo, Norway

1 In a randomized study of 22 patients in a maternity ward, the residual concentrations of two hypnotics, midazolam 15 mg p.o. and nitrazepam 5 mg p.o., in early breast milk and plasma were measured 7 h after intake on day 2 to day 6 postpartum. Milk pH, milk fat and binding to plasma proteins were also investigated. Sleep variables were scored on questionnaires.

2 No measurable ($< 10 \text{ nmol l}^{-1}$) concentrations of drug in milk were found in the group receiving 15 mg midazolam at night, either after the first night or after the fifth night. Additional investigations in two mothers demonstrated that midazolam and its hydroxy-metabolite disappeared rapidly from milk with undetectable levels after 4 h. The mean (s.d.) milk to plasma ratio for midazolam was 0.15 (0.06) in six paired samples. It may be assumed that practically no midazolam is transferred via early milk to the baby if the baby is nursed more than 4 h after tablet intake.

3 Milk nitrazepam concentrations increased significantly from the first (30 nmol l^{-1}) to the fifth morning (48 nmol l^{-1}) in the group receiving 5 mg nitrazepam at night. The mean (s.d.) milk to plasma ratio of nitrazepam after 7 h was 0.27 (0.06) in 32 paired samples, and did not vary from day 1 to day 5. Plasma protein binding of nitrazepam in puerperal women was found to be lower than that in plasma of healthy controls. The average amount of nitrazepam received by the breast-fed baby in the morning was calculated to increase from 1 to $1.5 \mu\text{g } 100 \text{ ml}^{-1}$ breast milk, from days 1 to 5. In the mothers nitrazepam was associated with better hypnotic effect, but a higher incidence of complaints than midazolam.

4 Milk pH, assuming anaerobic conditions, was found in 10 women to average 6.91 ± 0.09 (s.d.) on days 2–6 postpartum, which is less than previously reported.

5 It is concluded that both hypnotics may be used safely for a few days in the maternity ward. However, possible long-term effects in the suckling infant of small doses of benzodiazepines ingested with breast milk remain to be investigated.

Keywords nitrazepam midazolam breast milk milk fat milk pH plasma protein binding hypnotic effect

Introduction

In five Norwegian maternity wards hypnotics, predominantly nitrazepam, were given to 2/3 of women having vaginal deliveries (Matheson, 1985, 1989). Residual effects in the mother and

transfer of unknown amounts of hypnotics to neonates through breast milk are both matters of concern (Bennett *et al.*, 1988).

A previous study found relatively small

Correspondence: Dr I. Matheson, Department of Pharmacotherapeutics, University of Oslo, P.O. Box 1065 Blindern, N–Oslo 3, Norway

amounts of radiolabelled nitrazepam in breast milk (Rieder & Wendt, 1973). However, because a non-specific assay was used further studies using unlabelled drug were indicated.

Nitrazepam, which has a mean elimination half-life of 29 h, accumulates in plasma and causes impaired psychomotor function on the day after administration (Kangas & Breimer, 1981). Thus, a shorter acting hypnotic may be preferable for use in the maternity ward. Midazolam, having a mean half-life of 2.5 h, has very little residual effect (Dundee *et al.*, 1984; Godtliebsen *et al.*, 1986), but there is no information on its transfer into breast milk.

The aim of this study was firstly to compare the risk of accumulation in milk of nitrazepam and midazolam and to determine the amounts available to the baby breast-fed on the morning after single and repeated evening doses to the mother. Secondly, potential determinants of the passage of the drugs from plasma to breast milk in the early puerperal period, e.g. milk fat, milk pH and plasma protein binding were studied. Thirdly, hypnotic effects and maternal condition were evaluated after standard doses of the two hypnotics.

Methods

Subjects

Informed consent was obtained from 22 out of 70 eligible lactating women who had delivered healthy babies in the Department of Obstetrics and Gynecology, Ullevaal Hospital (Table 1). All of them had initiated breast-feeding and had produced about 15–20 ml milk at one feed. During the study concomitant medication was taken by 15 women (14 took paracetamol, one acetylsalicylic acid, two methylergometrine). Study day 1 usually corresponded to postpartum day 2.

One of the mothers and her baby were studied more extensively with regard to nitrazepam, as milk was also sampled at 11, 14 and 18 h on day 4, as well as at 11 h on day 3 and 5. Two

additional mothers had milk and blood samples drawn 0, 1, 2, 3, 4, 5, 6 and 7 h after administration of 15 mg midazolam at 2–3 months postpartum.

The study was approved by the Hospital Drug and Therapeutics Committee, and was notified to the Norwegian Medicines Control Authority.

Study design

Each mother was asked each night of the 5 day hospital period if she needed a sleeping pill. If so, she was then given either nitrazepam 5 mg ($n = 10$) or midazolam 15 mg ($n = 12$) in a double-blind random fashion. Intake of the tablet was checked each night and the exact time was noted. Venous blood samples (10 ml) and milk samples (3–8 ml) were taken each day around 06.00 h, i.e. about 7 h (range 6–8 h) after tablet intake and immediately before the morning feed. Milk was expressed manually and the first millilitre was discarded. An interval of 6–8 h since the previous breast feed was noted. Blood was centrifuged within 30 min and plasma and milk samples were frozen immediately at -20°C prior to assay.

Analysis of benzodiazepines in breast milk

The method of Berlin *et al.* (1972) was used with two extractions instead of one; the latter being an acid extraction using 5M HCl. After centrifugation, the acid phase was placed in an ice bath and 40% NaOH, phosphate buffer (pH = 7.0) and benzene were added. After mixing the benzene was evaporated almost to dryness and the residue was dissolved in 50 μl benzene containing flunitrazepam, as internal standard. Analysis was by gas chromatography with an electron capture detector. The retention times were 5, 3 and 4 min for nitrazepam, midazolam and hydroxy-midazolam, respectively. All analyses were done in duplicate. The plasma analysis was performed similarly with only one extraction. The limit of assay in milk was 10 nmol l^{-1} and the coefficient of intra-assay variation at 100 nmol l^{-1} was 4% for all three

Table 1 Characteristics of 22 mothers included in the study

	Nitrazepam ($n = 10$)	Midazolam ($n = 12$)
Mean age (range) (years)	28 (22–30)	27 (18–33)
Mean weight (range) (kg)	65 (55–75)	69 (62–75)
Primipara (n)	5	10
Caesarean/vaginal delivery (n)	1/9	2/10
Previous hypnotic use (n)	0	5

compounds. Inter-assay variation was 5.8%, 5.3% and 5.7% for nitrazepam, midazolam and hydroxy-midazolam, respectively.

The latter is an active metabolite of midazolam (Ziegler *et al.*, 1983). Owing to the high fat content of milk, two extractions were necessary giving a mean recovery of 80% for nitrazepam and 72% for both midazolam and its metabolite. By increasing the injection volumes the assay limit for midazolam was lowered to 5 nmol l⁻¹ in milk.

Milk fat

Breast milk was incubated with lipase and triglycerides were measured by a modification of an enzymatic method using a commercial kit for serum triglycerides and a TechniconTM Auto-analyser (Matheson *et al.*, 1990a). The coefficient of inter-assay variation was 5% ($n = 15$).

Milk pH

Breast milk was drawn directly from the nipples into thin capillary tubes, which were sealed with wax and placed in an ice bath. pH was measured within 30 min at 37° C by the Astrup technique using a capillary glass electrode (Radiometer G299A). The coefficient of variation of repeated estimates was 2% ($n = 15$).

Plasma protein binding of nitrazepam

Plasma (700 µl) containing [³H]-labelled and unlabelled nitrazepam was dialysed against buffer (700 µl Krebs-Ringer bicarbonate) in a thermostatted (37° C) shaker-incubator under an atmosphere of 5% CO₂ in air, thereby keeping plasma pH at 7.4 to 7.5 (Pike & Skuterud, 1983). Perspex dialysing cells and a 20/32 dialysis membrane (Union Carbide Corp. Chicago) were used. [³H]-nitrazepam (Amersham) with a specific activity of 0.45 mCi mmol⁻¹ was purified by t.l.c. using ethyl-acetate : methanol : 25% v/v ammonia (85:10:5) as the solvent system. The radiochemical purity was 98%. Nitrazepam was dissolved in ethanol (0.25 mmol l⁻¹ plasma). Pilot experiments showed that binding equilibrium was reached after 5 h. The percentage of unbound drug was calculated by measuring the ratio between the radioactivity (Wallac liquid scintillation counter) in buffer and plasma. Binding was independent of total drug concentration within the observed range. The day-to-day variation in estimates of binding was 4% ($n = 8$).

Plasma albumin and α₁-acid glycoprotein (AAG) were assayed using the bromocresol purple

method (Pinnel & Northam, 1978) and a nephelometric technique (Sternberg, 1977), respectively.

Evaluation of hypnotic effect

A total sleep index was calculated for each patient based on four sleep variables (onset, awakenings, quality, morning condition) scored by a self-report questionnaire (Godtliebsen *et al.*, 1986; Hindmarch *et al.*, 1984). Sedation and side effects were also evaluated. Comparisons were made with Student's *t*-test and the Wilcoxon rank sum test.

Results

Transfer of nitrazepam into milk

Corresponding milk and plasma drug concentrations are shown in Figure 1; the mean (s.d.) milk to plasma ratio at 7 h was 0.27 (0.06) ($n = 9$). Owing to reservations about the procedures the number of mothers studied varied from day to day. Only three mothers in each group completed all 5 study days. The accumulation ratios between study days 1 and 5 were 1.5 and 1.6 in plasma and milk, respectively. One mother (number 2) did not produce sufficient milk to measure nitrazepam. Mean values on days 3, 4 and 5 postpartum for milk/plasma (M/P) ratio, milk fat, unbound drug fractions in plasma and the concentrations of plasma proteins are summarised in Table 2. Significant differences between day 3 and day 5 were found only for

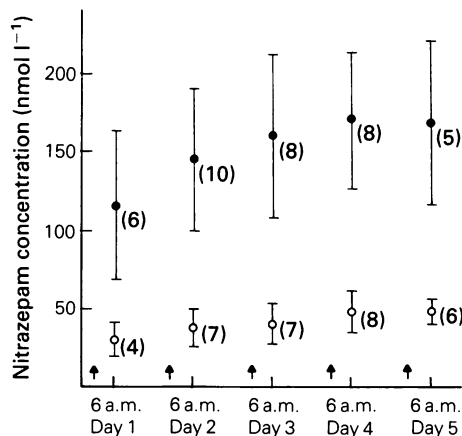


Figure 1 Mean \pm s.d. nitrazepam concentration in plasma (\bullet) and milk (\circ) from puerperal women after 5 mg nitrazepam (\uparrow) for 5 nights postpartum. Number of subjects in parentheses. The detection limit was 10 nmol l⁻¹.

Table 2 Day-to-day variation in milk and plasma characteristics in nine puerperal women after intake of 5 mg nitrazepam. Differences between day 3 and day 5 tested by paired *t*-test. *n* = total number of observations

Variable	Days postpartum			Total mean \pm s.d.	(n)	Day 3–Day 5 P
	3	4	5			
Milk/plasma ratio	0.268	0.286	0.290	0.283 \pm 0.06	(27)	NS
Plasma unbound fraction	0.192	0.201	0.181	0.192 \pm 0.018	(27)	NS
Plasma albumin (g l ⁻¹)	25.4	28.4	27.5	27.3 \pm 3.9	(27)	NS
Plasma AAG (g l ⁻¹)	1.03	1.12	1.26	1.14 \pm 0.15	(23)	< 0.01
Milk triglycerides (g l ⁻¹)	16.9	21.7	20.1	19.9 \pm 4.5	(24)	NS

AAG = α_1 -acid glycoprotein.

plasma AAG. Mean M/P-ratios (range 0.18–0.53) varied significantly ($P < 0.001$, Kruskal Wallis test) between the nine mothers studied, but multiple regression analysis revealed no significant associations between the individual M/P-ratios and the other variables (Table 2) in this small number of women.

Based on the average morning milk drug concentration a breast-fed baby would receive 1 and 1.5 μ g NZ (MW = 281) per 100 ml feed on day 1 and day 5, compared with 2 μ g per 100 ml if the highest milk concentration observed was assumed. Milk drug concentrations in one mother indicated small variations during day time, as seen in Figure 2. On day 5 no measurable (< 10 nmol l⁻¹) nitrazepam was detected in the plasma of one infant whose mother took the hypnotic on 5 consecutive nights.

Milk pH and triglycerides

The mean \pm s.d. pH of milk from women ($n = 10$) who took either nitrazepam or midazolam decreased gradually, being 7.09 ± 0.16 , $6.98 \pm$

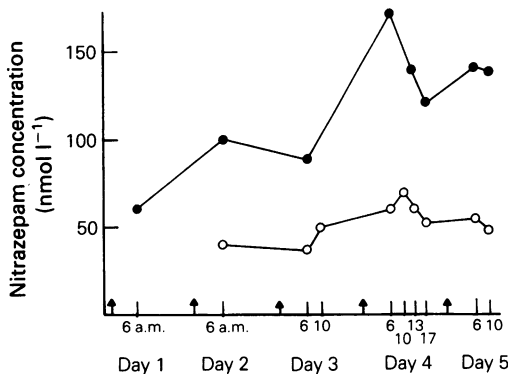


Figure 2 Nitrazepam concentrations in plasma (●) and milk (○) (M/P = 0.39 ± 0.03) from one mother (no 10/34 years/75 kg) during the first days postpartum. The detection limit was 10 nmol l⁻¹. (↑) 5 mg nitrazepam.

0.03, 6.90 ± 0.07 and 6.82 ± 0.08 on days 2, 3, 4 and 5 postpartum (*t*-test day 2–day 5, $P < 0.01$). No difference in the pH of milk from the two breasts was observed. The mean value for all days was 6.91 ± 0.09 . Milk triglycerides in individual samples ($n = 34$) at 06.00 h varied from 9–36 mmol l⁻¹. In 10 women the mean \pm s.d. value was 22.8 ± 1.6 before and 36.8 ± 10.2 after the morning feed.

Plasma protein binding

The unbound fraction of nitrazepam in plasma from the puerperal women varied between mothers but not between days. It was higher ($P < 0.005$) than that found in pooled plasma from healthy volunteers (0.186 ± 0.010 vs 0.153 ± 0.056 , $n = 8$). The plasma binding of nitrazepam was correlated with albumin concentration ($r = 0.38$), but not with that of AAG in plasma.

Transfer of midazolam into milk

No measurable drug (< 10 nmol l⁻¹) was detected in breast milk from 11 mothers the morning after the 15 mg dose. Only one mother had a detectable concentration of 30 nmol l⁻¹ (M/P = 0.20), reflecting accidental intake of an additional tablet of midazolam. Mean \pm s.d. plasma concentrations at 7 h in 30 samples were 32.1 ± 1.2 nmol l⁻¹ midazolam and 11.5 ± 3.7 nmol l⁻¹ hydroxy-midazolam with no increases upon repeated doses.

Milk and plasma concentration-time curves of midazolam and hydroxy-midazolam in two mothers at a later postpartum stage are shown in Figure 3a and b. In six paired samples the M/P-ratio was found to be 0.15 ± 0.06 . Calculation based on areas under the curve gave M/P AUC values of 0.09 and 0.16. Based on average concentrations in milk (10 nmol l⁻¹) a breast-fed baby would receive 0.33 μ g midazolam (MW = 325) and 0.34 μ g of the metabolite per 100 ml breast milk.

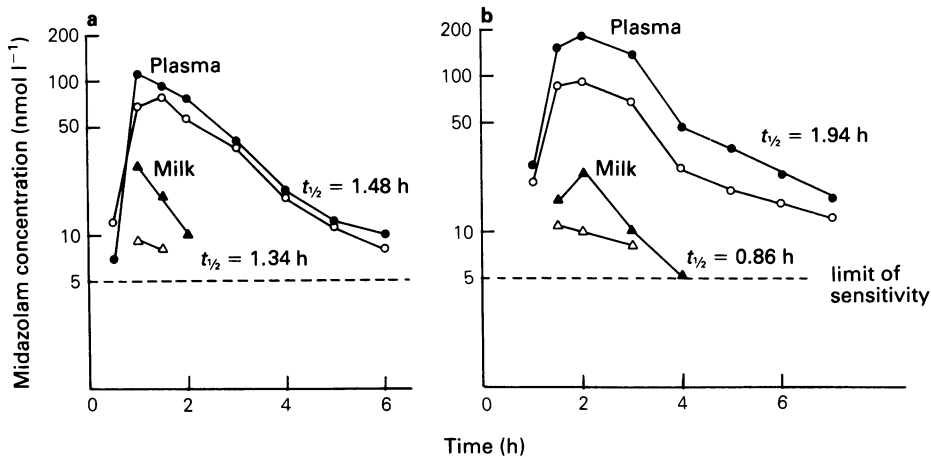


Figure 3 a and b. Midazolam (●, ▲) and hydroxymidazolam (○, △) concentrations in plasma and milk from two women after 15 mg midazolam at 2–3 months postpartum. The detection limit was 5 nmol l⁻¹.

Clinical effects

Nitrazepam was associated with significantly fewer awakenings ($P < 0.01$) and a higher total sleep index ($P < 0.05$) than midazolam.

Each sleep variable was also assessed for each night and a significant difference in favour of nitrazepam was found for awakenings the first night. There was no difference in morning sedation. Four complaints (two of headache, two of dizziness) in the nitrazepam group and none in the midazolam group were recorded up to 1 h after waking up. The total duration of sleep varied between 6 and 7 h after administration of both drugs.

Discussion

For ethical and practical reasons the timing of samples was limited by ward routine such that the mothers slept through the night and the babies were not breast-fed before 06.00 h (sucrose solution was given at night). None of the mothers complied with the intention to get a milk sample if they woke up at night. Thus, pharmacokinetic comparisons in milk were not possible as nitrazepam could be measured only after 7 h, at a time when midazolam was undetectable.

Milk transfer

The observed M/P ratio for a particular drug varies with the time since administration, the number of doses, the stage of lactation, the milk fat content, and the milk pH (Bennett *et al.*,

1988; Wilson *et al.*, 1980). In the case of nitrazepam the milk and plasma elimination curves were not described (Figure 1). Hence, it is not known whether the observed M/P ratio equals the true M/P value based on AUCs. However, indirect evidence from our findings indicates that equilibrium was obtained at 7 h. Thus, the M/P values observed within individuals remained constant from day to day, the drug is lipid-soluble and it is slowly eliminated from plasma and, in one mother, only small variations in M/P-values were observed at different times after drug intake (Figure 2). Moreover, for another hypnotic, zopiclone, M/P-ratios based on paired samples and AUCs were closely correlated (Matheson *et al.*, 1990b).

The lack of correlation between milk triglyceride concentration and M/P-values of lipid soluble nitrazepam ($\log P = 2.12$) is noteworthy. We did not measure the binding of nitrazepam to milk components in this study, but estimates based on the observed plasma-binding suggest only 1% binding (Atkinson & Begg, 1988). Recent *in vitro* studies found that lactational stage had a minimal effect on the predicted M/P-ratio of diazepam and propranolol (Fleishaker *et al.*, 1989). However, changes in total plasma drug concentrations that might result from plasma binding changes were not considered.

The accumulation of drug in milk may represent a risk for compounds with long half-lives (Needs *et al.*, 1985). Accordingly, we observed 60% higher milk concentrations of nitrazepam on day 5 compared with day 1, a period which is considered sufficient to establish steady state plasma drug concentrations (Kangas & Breimer, 1981). There was no tendency to a

higher accumulation in milk than could be derived from plasma data. The dose to a breast-fed infant would still be low and probably without effect even though a physiological role of similar doses of benzodiazepine-like substances in breast milk was recently postulated (Dencker & Johansson, 1990). Assuming that unchanged nitrazepam represents 50% of the total radioactivity, the milk concentrations observed previously (Rieder & Wendt, 1973), would be 2–3 times higher than ours under similar study conditions. Others (Kangas & Breimer, 1981) have reported plasma concentrations of nitrazepam similar to ours.

The mean plasma concentration of midazolam in our mothers was similar to that reported previously in women at parturition (Kanto *et al.*, 1983).

Plasma protein binding of nitrazepam

Two previous studies (Jochemsen *et al.*, 1982; Rieder & Wendt, 1973) reported slightly lower unbound fractions of nitrazepam (0.136 and 0.123), than that in our healthy controls and markedly lower than that in our lactating mothers. It may be assumed that puerperal women have a reduced binding capacity for nitrazepam due either to haemodilution with subsequent lowered (Dean, 1980; Notarianni, 1990) plasma albumin or to the presence of endogenous displacers, e.g. increase in free fatty acids (Burt, 1960). The observed moderate increase in plasma AAG level in the puerperal period confirms recent findings (Fleishaker *et al.*, 1989; Norris *et al.*, 1990).

Milk pH

In previous studies puerperal milk pH measured aerobically was reported to be 7.30 and 7.10 (Harrison & Peat, 1972; Morriss *et al.*, 1986). The lower value (6.91) observed in the present study may be due to the observation that once the wax was removed and contact with air was established, the pH increased by 0.1 or 0.2 units during the period of measurement. Like Morriss *et al.* (1986) we observed a tendency to more acidic milk at the end of the first week post-partum. This finding may confound some of the M/P-ratios calculated by the equations proposed

by Atkinson & Begg (1988) and Fleishaker *et al.* (1989).

Nitrazepam and midazolam as hypnotics

Comparisons between hypnotics with widely different kinetics are difficult. The choice of drug dosage and the battery of sleep variables will influence the hypnotic effect measured. In contrast to a previous study (Godtlibsen *et al.*, 1986) using the same standard doses in volunteers we demonstrated a higher number of awakenings with midazolam compared with nitrazepam. Rebound insomnia, considered to be a problem with short-acting benzodiazepines (Bixler *et al.*, 1985), was not assessed in our study.

Conclusions

The most important question is whether hypnotics are needed by healthy women having normal deliveries. In many hospitals maternity ward routines include breast-feeding at night in order to improve milk yield. In terms of hypnotic drug intake conflicting interests arise on the mother's side, including the dilemma that drug exposure of the infant would then be substantially higher than that estimated in this study. However, since the milk intake of babies is very limited during the first days after birth, the amount of drug transferred through milk remains small. Further diminution of that amount is possible by using maternal doses of 2.5 mg nitrazepam and 7.5 mg midazolam whenever effective. Moreover, potential long-term effects of receiving small doses of hypnotics during a vulnerable period of life, have not been investigated. In general, it seems prudent to restrict the use of hypnotics to those drugs with the lowest transfer and to confine administration to those patients who really need a hypnotic.

Our special thanks are due to Hans A. Sande for allowing access to patients in the maternity ward. The advice and assistance of Eva Pike, Ludvig Daae and Kirsten Midtbøen Olsen are appreciated.

I. Matheson was a research fellow of the Norwegian Research Council for Science and the Humanities. We thank all the mothers who contributed to the study and Hoffmann-La Roche, Oslo, for supply of the drugs.

References

- Atkinson, H. C. & Begg, E. J. (1988). Prediction of drug concentrations in human skim milk from plasma protein binding and acid-base characteristics. *Br. J. clin. Pharmacol.*, **25**, 495–503.
- Bennett, P. N., Matheson, I., Dukes, M. N. G. *et al.* (eds) (1988). *Drugs and human lactation*. Amsterdam: Elsevier.

- Berlin, A., Siwers, B., Agurell, S., Hiort, A., Sjöqvist, F. & Strom, S. (1972). Determination of bioavailability of diazepam in various formulations from steady state plasma concentration data. *Clin. Pharmac. Ther.*, **13**, 733-744.
- Bixler, E. O., Kales, J. D., Kales, A., Jacoby, J. A. & Soldatos, C. R. (1985). Rebound insomnia and elimination half life: assessment of individual subject response. *J. clin. Pharmac.*, **25**, 115-124.
- Burt, R. L. (1960). Plasma nonesterified fatty acids in normal pregnancy and puerperium. *Obstet. Gynecol.*, **15**, 460-464.
- Dean, M., Patterson, R. J. & Levy, G. (1980). Serum protein binding of drugs during and after pregnancy in humans. *Clin. Pharmac. Ther.*, **28**, 253-261.
- Dencker, S. J. & Johansson, G. (1990). Benzodiazepine-like substances in mother's milk (letter). *Lancet*, **i**, 413.
- Dundee, J. W., Halliday, N. J., Harper, K. W. & Brogden, R. N. (1984). Midazolam. A review of its pharmacological properties and therapeutic use. *Drugs*, **28**, 519-543.
- Fleishaker, J. C., Desai, N. & McNamara, P. J. (1989). Possible effect of lactational period on the milk-to-plasma drug concentration ratio in lactating women: Results of an *in vitro* evaluation. *J. pharm. Sci.*, **78**, 137-141.
- Godtliebsen, O. R., Jerkø, D., Gordeladze, J. O., Bredesen, J. E. & Matheson, I. (1986). Residual effect of single and repeated doses of midazolam and nitrazepam in relation to their plasma concentrations. *Eur. J. clin. Pharmac.*, **29**, 595-600.
- Harrison, V. C. & Peat, G. (1972). Significance of milk pH in newborn infants. *Br. med. J.*, **4**, 515-518.
- Hindmarch, I., Ott, H. & Roth, T. (eds) (1984). *Sleep, benzodiazepines and performance. Experimental methodologies and research prospects*. Berlin: Springer-Verlag.
- Jochimsen, R., Hogendoorn, J. J. H., Dingemans, I. *et al.* (1982). Pharmacokinetics and bioavailability of intravenous, oral and rectal nitrazepam in humans. *J. Pharmacokin. Biopharm.*, **10**, 231-245.
- Kangas, L. & Breimer, D. D. (1981). Clinical pharmacokinetics of nitrazepam. *Clin. Pharmacokin.*, **6**, 346-366.
- Kanto, J., Sjøvall, S., Erkkola, R., Himberg, J.-J. & Kangas, L. (1983). Placental transfer and maternal midazolam kinetics. *Clin. Pharmac. Ther.*, **33**, 786-791.
- Matheson, I. (1985). Drugs taken by mothers in the puerperium (Letter). *Br. med. J.*, **290**, 1588-1589.
- Matheson, I. (1989). Drugs to mother and infant in the maternity ward. A survey in five Norwegian university hospitals. (In Norwegian). *Tidsskr. Nor. Lægeforen*, **109**, 2118-2122.
- Matheson, I., Foss, O. P. & Skaare, J. U. (1990a). Fat content and xenobiotic concentrations in human milk: Validation of an enzymatic assay of milk fat. *Pharmacology & Toxicology*, (in press).
- Matheson, I., Sande, H. A. & Gaillot, J. (1990b). The excretion of zopiclone into breast milk. *Br. J. clin. Pharmac.*, **30**, 267-271.
- Morriss, F. H., Brewer, E. D., Spedale, S. B. *et al.* (1986). Relationship of human milk pH during course of lactation to concentrations of citrate and fatty acids. *Pediatrics*, **78**, 458-464.
- Needs, C. J. & Brooks, P. M. (1985). Antirheumatic medication during lactation. *Br. J. Rheumatol.*, **24**, 291-297.
- Norris, M. C., Ala-Kokko, T. I., Leighton, B. A. & DeSimone, C. (1990). Serum concentrations of alpha₁-acid glycoprotein and albumin following cesarean section and vaginal delivery. *Drug Int. clin. Pharmac.*, **24**, 328-329.
- Notarianni, L. J. (1990). Plasma protein binding of drugs in pregnancy and in neonates. *Clin. Pharmacokin.*, **18**, 20-36.
- Pike, E. & Skuterud, B. (1983). Methodological aspects on the determination of plasma binding of amitriptyline and nortriptyline. *Nor. Pharm. Acta*, **45**, 33-42.
- Pinnel, A. H. & Northam, B. E. (1978). New automated dye-binding method for serum albumin determination with bromocresol purple. *Clin. Chem.*, **24**, 80-86.
- Rieder, J. & Wendt, G. (1973). Pharmacokinetics and metabolism of the hypnotic nitrazepam. In *The benzodiazepines*, eds Garattini, S., Mussini, E. & Randall, L. O., pp. 99-127. New York: Raven Press.
- Sternberg, I. C. (1977). A rate nephelometer for measuring specific proteins by immunoprecipitation reactions. *Clin. Chem.*, **23**, 1456-1464.
- Wilson, J. T., Brown, R. D., Cherek, D. R. *et al.* (1980). Drug excretion in human breast milk. Principles, pharmacokinetics and projected consequences. *Clin. Pharmacokin.*, **5**, 1-66.
- Ziegler, W. H., Schalch, E., Leishman, B. & Eckert, M. (1983). Comparison of the effects of intravenously administered midazolam, triazolam and their hydroxymetabolites. *Br. J. clin. Pharmac.*, **16**, 63S-69S.

(Received 29 May 1990,
accepted 9 July 1990)