The Placental and Mammary Transport of $[^{14}\mathrm{C}]$Menaquinone-4 in Rats

Kyoichi TADANO, Teruaki YUZURIHA, and Yasuo MIYAKE

Tsukuba Research Laboratories, Eisai Co., Ltd., Tokodai, Tsukuba 300-26, Japan
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Summary The transfer of menaquinone-4 (vitamin K$_{2(20)}$) to the fetus and milk was studied in pregnant and lactating rats, respectively, after oral administration (4 mg/kg) of $[^{3\alpha-14}\mathrm{C}]$menaquinone-4. Intestinal absorption of menaquinone-4 was rapid and the highest level of radioactivity in each tissue except guts of fetal rats was observed at 4 h after dosing. The level in the fetal homogenate was low. At that time, the concentration of menaquinone-4 in the fetal liver was 84 ng/g, corresponding to 9% of the value found in the placenta. Therefore, we conclude that the transfer of menaquinone-4 to the developing rat fetus is restricted by the blood-placenta barrier, but that a sufficient amount of menaquinone-4 (more than the essential amount of vitamin K to ensure full carboxylation) can be transferred into the fetal liver. It was also observed that the radioactivity was transferred to milk after oral administration to lactating rats. Milk/blood concentration ratios at 6 and 24 h after dosing were 13.8 and 65.1, respectively. The elimination half-life of radioactivity in milk was about 17 h. Eighty-four percent of milk radioactivity was due to menaquinone-4. These results suggest that the prophylactic maternal oral administration of menaquinone-4 may be efficacious for a prophylaxis of neonatal and infantile vitamin K deficiency.

Key Words menaquinone-4, placental transport, mammary transport, rat, fetus, vitamin K deficiency, vitamin K$_{2(20)}$

It is well known that vitamin K deficiencies cause gastrointestinal and intracranial hemorrhage in newborn and young infants (1, 2). These disorders occur more frequently in breast-fed babies than in bottle-fed babies. Therefore breast-feeding is said to be a major risk factor of vitamin K deficiency, but the reason has not yet been fully clarified. Recently, some clinical studies have indicated that oral administration of vitamin K to the mother during the last stage of pregnancy is useful to protect babies from vitamin K deficiency (3, 4).

The vitamin K group includes several homologs which differ in the alkyl side chain at the 3-position of the naphthoquinone ring. Of these homologs,
menaquinone-4 (vitamin K \(_{2(20)}\)) is considered to be a physiologically active form of this vitamin (5). However, no animal study has yet been done on the transfer of menaquinone-4 and its metabolites to the fetus and milk in female animals, in contrast to phylloquinone. We have therefore studied the placental and mammary transport of \([^{14}C]\)menaquinone-4 in pregnant and lactating rats after oral administration of the vitamin, in order to evaluate the efficacy of prophylactic maternal administration of menaquinone-4 for neonatal and infantile vitamin K deficiency. The results of these experiments are presented in this paper.

MATERIALS AND METHODS

1. Chemicals. \([3^{-14}C]\)Menaquinone-4 (Fig. 1) was synthesized in our laboratories (by Dr. Hamamura). The specific activity was 1.50 MBq/mg (40.55 µCi/mg) and the radiochemical purity was greater than 98% based upon thin-layer chromatography. The percentages of trans-form and cis-form were 96 and 4, respectively.

All other chemicals and reagents were of analytical grade and were used without further purification.

2. Preparation of \([^{14}C]\)menaquinone-4 solution. \([^{14}C]\)Menaquinone-4 (10 mg) and HCO-60 (70 mg) were taken into a low actinic culture tube and heated on a water bath (80–85°C) for a few minutes. Then about 2 ml of hot distilled water (90–95°C) was added and the solution was sonicated for 10 min with an ultrasonic cleaner (Branson Model B12). Finally, the total volume of the solution was adjusted to 2.5 or 10 ml with hot distilled water. The procedures were all carried out in a dark room to prevent photo-degradation of menaquinone-4.

3. Animal experiment. Pregnant and lactating rats of the Wistar strain (specific pathogen-free) were purchased from Shizuoka Laboratory Animal Center (Japan). Rats were fed commercial diet (Oriental Yeast Co., Ltd., Tokyo) and environmental conditions were controlled at all times (relative humidity, 55±5%; temperature, 23±0.5°C).

1) Oral administration to pregnant animals: Pregnant rats weighing 190–225 g were used on day 13 or 19 of gestation and they were fasted for 16 h prior to dosing.

\([^{14}C]\)Menaquinone-4 was given orally to the rats by gastric intubation at a dose of 4 mg/kg/4 ml. After dosing, blood samples (50 µl) were collected at fixed times from the tail vein. At 1, 4, or 24 h after administration, animals were killed by bleeding from the inferior aorta under ether anesthesia and the blood was

![Fig. 1. Chemical structure and \(^{14}C\)-labeled position (*) of menaquinone-4.](image-url)
collected in heparinized tubes. The various tissues as well as the fetuses were removed immediately and washed with ice-cold saline.

Whole-body autoradiography. Rats were killed under anesthesia with ether at 1, 4, and 24 h after single oral administration, and immediately frozen in dry-ice hexane. Fifty µm sections of the frozen samples were obtained with a PMV Cryo-Microtome (2250, LKB-Produkter AB) according to Ullberg's method (6), and attached to Salotape (Hisamitsu Pharmaceutical Co., Ltd., Saga). The sections were then freeze-dried and were placed in contact with X-ray films (New A, Konica Co., Ltd., Tokyo) for 6 weeks. The exposed film was developed with Konidol (Konica) for 4 min and fixed with Konifix (Konica) for 4 min at 20°C.

2) Intravenous administration to pregnant animals: Pregnant rats weighing 210–230 g were used on day 19 of gestation and they were fasted for 16 h prior to dosing. [14C]Menaquinone-4 was given intravenously to rats at a dose of 4 mg/kg/ml. At 4 h after dosing, the animals were killed and the various tissues were removed in the same manner as described in the case of oral administration.

3) Oral administration in lactating animals: Lactating rats weighing 200–230 g were used on the 14th day after delivery and were not fasted before or during the experiment. [14C]Menaquinone-4 was given orally to dams at the same dose as in the case of pregnant rats. At 1, 3, 6, 24, and 53 h after dosing, sucklings that had been removed from their dams for 2 h were allowed to suckle from their respective dams. Then the sucklings were killed by decapitation and blood and semisolid milk in the stomach were collected. At the same time, blood samples (50 µl) were collected from the tail vein of the dams.

4. Measurement of radioactivity and determination of metabolites. A 50 µl aliquot of blood was solubilized with 0.75 ml of Soluene-350 (Packard)/isopropanol (1:1, v/v), and then several drops of 30% H₂O₂ were added to the solubilized blood samples for decolorization.

In the case of the milk and tissues, about 50 mg of each sample was incubated with 0.5 ml of Soluene-350 at 50°C for 2 h. Next, 5 ml of scintillation fluid consisting of Insta-Gel (Packard)/0.5 N HCl (9:1, v/v) was added to each solubilized sample, and the radioactivity in each sample was measured with a liquid scintillation counter (LSC-903, Aloka). The quenching level was corrected by employing an automatic external standard method.

Plasma and tissue homogenates were adjusted to pH 2–3 with 2 N HCl and extracted twice with 3 vol. of tetrahydrofuran/ethyl ether (1:1, v/v). The extracts were evaporated to dryness and the residues were subjected to TLC in solvent systems I (benzene only) and II (cyclohexane/ethyl ether/ethyl alcohol; 16:4:3 by vol.)

The pooled samples (3–6 h and 24 h after dosing) of milk were homogenized and extracted twice with 30 ml of ethyl acetate/ethyl ether (2:1, v/v). The organic phase was concentrated and chromatographed over a column of silica gel with n-hexane (first effluent) and n-hexane/ethyl acetate (1:1) (second effluent). The second effluent was concentrated and submitted to TLC with solvent systems I and II.

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The radioactive spots were detected by TLC-autoradiography and the amounts of menaquinone-4 and its metabolites were measured with a Berthold LB2832 automatic TLC linear analyzer.

RESULTS

Disposition in the Maternal-Fetal Unit

Blood levels. Figure 2 shows the blood level of the radioactivity after oral administration of [14C]menaquinone-4 to pregnant rats on day 19 of gestation. The radioactivity rapidly appeared in the circulation, reaching the peak concentration at 1.2 h after dosing, then disappearing quickly. These data prove that the intestinal absorption of menaquinone-4 is rapid.

Whole-body autoradiography. The overall distribution of radioactivity was determined by whole-body autoradiography. Figure 3 shows the whole-body autoradiograms prepared 1, 4, and 24 h after oral administration of [14C] menaquinone-4 to pregnant rats on day 13 of gestation. At 1 h, high radioactivity was detected in the gastrointestinal contents and liver of the maternal rat, but was not observed in the fetuses. The radioactivity in the amnion and placenta was slightly lower than that in maternal blood. At 4 h, the highest radioactivity was observed in the maternal liver and upper intestinal contents. The radioactivity levels in the adrenal, heart, and brown fat were highest, after that in the liver. At this time, the fetus as well as amnion and placenta showed radioactivity. At 24 h after

![Fig. 2. Blood level of radioactivity in pregnant rats after oral administration of [14C]menaquinone-4 (4 mg/kg) on day 19 of gestation (n=3).](image-url)
administration, the radioactivity was decreased all over the body except in the intestinal and colonic contents. High radioactivity in them indicates that menaquinone-4 is mainly excreted via the bile.

Figure 4 shows the whole-body autoradiograms after administration of the labeled compound to pregnant rats on day 19 of gestation. At 1 h after administration, the radioactivity was found all over the body except the fetus, and the level was especially high in the gastrointestinal contents, liver, heart, maternal blood, and placenta. The highest radioactivity at 4 h after administration was found in maternal liver. At this time, the fetus, amnion, and placenta showed radioactivity and the fetal liver showed prominent activity. At 24 h after administration, the radioactivity in each tissue except the intestinal contents was reduced similarly to the case of pregnant rats on day 13 of gestation.

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Fig. 4. Whole-body autoradiograms showing the distribution of radioactivity at 1, 4, and 24h after oral administration of \([^{14}C]\)menaquinone-4 (4 mg/kg) to pregnant rats on day 19 of gestation. White areas correspond to radioactivity.

The pattern of distribution in the main organs of pregnant rats was parallel with that of male rats (7).

_Tissue levels._ Figure 5 shows the distribution of radioactivity (µg menaquinone-4 equivalent/g or ml) in various tissues after oral administration of \([^{14}C]\)menaquinone-4 to pregnant rats on day 13 of gestation. At 1 and 4h, the maternal liver and adrenal had much higher levels of radioactivity than the other tissues. In fetuses, the radioactivity increased as time passed, but the concentration in the fetus was as low as 1/20 to 1/3 of that of the placenta. This suggests that the transfer of menaquinone-4 to the fetus is restricted by the blood-placenta barrier.

Figure 6 shows the distribution of radioactivity in maternal and fetal tissues after oral administration of \([^{14}C]\)menaquinone-4 to pregnant rats on day 19 of gestation. At 1 and 4h, the highest radioactivity was detected in the maternal
liver. At 4 and 24 h, the radioactivity in the fetus and the ratio of the concentration in the fetus to that in the placenta were higher than those in pregnant rats on day 13 of gestation. The radioactivity in mammary gland remained at a high level, which suggests that menaquinone-4 is easily transferred to milk. At 1, 4, and 24 h after dosing, the radioactivity found in a fetus amounted to 0.003, 0.027, and 0.03% of the dose, respectively. In the fetal tissues, the concentrations of radioactivity in liver and guts were higher than those in blood and brain. The concentration of radioactivity in fetal liver was 31.7% and 101.9% of that in placenta at 4 and 24 h after dosing, respectively.

Table 1 shows the concentration of menaquinone-4 and the percent of unchanged form with respect to total radioactivity in each sample at 4 h after oral or intravenous administration. The concentration of menaquinone-4 in placenta after oral dosing was 0.921 µg/g and that in fetal liver was 0.084 µg/g. In fetal liver,
Fig. 6. Tissue distribution of radioactivity in pregnant rats (left) and fetuses (right) after oral administration of $^{14}$C-menaquinone-4 (4 mg/kg) on day 19 of gestation.

Table 1. Concentration of menaquinone-4 and the percent of unchanged form with respect to total radioactivity in each sample at 4 h after oral or intravenous administration of $^{14}$C-menaquinone-4 (4 mg/kg) to pregnant rats on day 19 of gestation.

| Sample   | Concentration (µg/g) | % of total radioactivity | Concentration (µg/g) | % of total radioactivity |
|----------|----------------------|--------------------------|----------------------|--------------------------|
| Plasma   | 1.537 (1.7)          | 80.4                     | 0.747 (0.3)          | 12.8                     |
| Liver    | 17.282 (18.8)        | 68.9                     | 10.025 (4.5)         | 65.9                     |
| Placenta | 0.921 (1.0)          | 82.1                     | 2.230 (1.0)          | 78.7                     |
| Fetal liver | 0.084 (0.1)        | 38.8                     | 0.291 (0.1)          | 44.7                     |

Each value represents the mean of 3 animals. The values in brackets show the ratios to the concentration of menaquinone-4 in placenta.

the percent of menaquinone-4 with respect to total radioactivity in the sample was low (38.8%) in comparison with maternal plasma (80.4%), liver (68.9%), and placenta (82.1%). The concentrations of menaquinone-4 in placenta and fetal liver
after intravenous administration were about 3 times greater than those after oral administration. On the other hand, the concentration ratio of menaquinone-4 in placenta to that in fetal liver was the same (10:1) in both cases. These results suggest that the concentration of menaquinone-4 in fetal liver depends on that in placenta.

Transfer to milk

Table 2 shows the milk and maternal blood levels of radioactivity and ratio of milk/blood levels after oral administration of \([^{14}\text{C}]\text{menaquinone-4} (4\text{mg/kg})\) to lactating rats. The milk level was lower than the blood level at 1h after dosing. However, the milk level increased until 6–24h after administration and remained high thereafter, whereas the blood level decreased rapidly from 1h. The concentration of radioactivity in milk was 3.0, 13.8, 65.1, and 31.6 times higher than that in maternal blood at 3, 6, 24, and 53h, respectively. These results indicate that \([^{14}\text{C}]\text{menaquinone-4}\) is easily transferred into milk. The elimination half-life of milk radioactivity was found to be 17.2h.

The blood levels of radioactivity in sucklings are shown in Table 3. It seems

| Time (h) | Concentration (\(\mu\text{g Eq.}/\text{g or ml}\)) | Milk/blood level ratio |
|---------|---------------------------------|----------------------|
|         | Milk (n=6) | Blood (n=3) |                      |
| 1       | 0.03±0.02 | 1.32±0.08 | 0.03±0.02 |
| 3       | 1.09±0.38 | 0.33±0.03 | 3.04±1.54 |
| 6       | 1.82±0.42 | 0.13±0.01 | 13.80±4.13 |
| 24      | 1.82±0.09 | 0.03±0.00 | 65.13±3.45 |
| 53      | 0.61±0.05 | 0.02±0.00 | 31.63±5.08 |

Each value represents the mean±SE.

Table 3. Blood level of radioactivity in sucklings after oral administration of \([^{14}\text{C}]\text{menaquinone-4} (4\text{mg/kg})\) to lactating rats.

| Time (h) | Concentration (\(\mu\text{g}\) menaquinone-4 Eq./ml) |
|---------|-------------------------------------------------|
| 1       | 0.000±0.000                                      |
| 3       | 0.001±0.000                                      |
| 6       | 0.014±0.001                                      |
| 24      | 0.029±0.002                                      |
| 53      | 0.003±0.000                                      |

Each value represents the mean±SE of 6 sucklings.

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clear that menaquinone-4 is absorbed through the milk in sucklings and transferred into the systemic circulation.

Menaquinone-4 level was determined with approximately 2 g of the milk at 3–6 h and 24 h after oral administration. After extraction with ethyl acetate/ethyl ether (2:1) and column chromatography on silica gel, 94.5% of the radioactivity in milk passed into the second effluent fraction in both samples and all that of unchanged form was included in this fraction. Figure 7 shows the TLC-radiochromatograms and autoradiograms of the second effluent fraction. On these radiochromatograms, unchanged form accounted for 89.0% of the radioactivity applied to TLC, respectively. So it was found that 84.1% of the radioactivity in both milk samples was due to menaquinone-4 and the rest was due to polar metabolites of menaquinone-4. Therefore, menaquinone-4 is mostly transferred intact into milk.

**DISCUSSION**

When [14C]menaquinone-4 was administered orally to pregnant rats, the blood level of radioactivity reached a peak at 1.2 h after dosing and rapidly declined thereafter (Fig. 2). We observed rapid intestinal absorption of menaquinone-4 and a quick decline of radioactivity in the blood, similar to the case of phylloquinone in previous studies (8, 9) using the normal rat. But intestinal absorption of vitamin K by humans is slower both in the child (10) and in the adult (11–13).

After oral administration of [14C]menaquinone-4 to maternal rats, unchanged form was transferred to the fetus, though in small quantities. Transfer of [14C]menaquinone-4 to the developing rat fetus was restricted by the blood-placenta.
barrier as judged from the maternal and fetal concentration ratios and the results of whole-body autoradiography of pregnant rats at various times after dosing.

The first indication of the existence of a placental barrier for phylloquinone was reported by Shearer et al. (14). In human volunteers, they could not detect phylloquinone in cord blood, which confirms that the vitamin level is very low at birth. Recently, Hamulyák et al. (15) and Guillaumont et al. (16) reported that the placental transport of phylloquinone in pregnant rats was very low, and concluded that a substantial placental barrier existed to the transport of pharmacologically significant amounts of the vitamin. Our result for menaquinone-4 is consistent with this.

In the fetal tissues after oral administration of $[^{14}C]$menaquinone-4 to pregnant rats on day 19 of gestation, the concentrations in liver and guts were higher than those in other tissues. The concentration of radioactivity in fetal liver was 31.7% and 101.9% of that in placenta at 4 and 24 h after dosing, respectively (Fig. 6). At 4 h after dosing, the concentration of radioactivity in fetal liver was 216 ng Eq./g and that of unchanged menaquinone-4 was 84 ng/g (Table 1), which is substantially more than the essential quantity of vitamin K (proposed to be 4.5 ng/g by Taggart and Matshiner (17), and 8-44 ng/g by Haroon and Hauschka (18) required for maintenance of the physiological function of the liver.

The fetal liver contained a relatively large amount of menaquinone-4 metabolites (Table 1), indicating that the blood-placenta barrier for these metabolites is low, and/or more rapid degradation of menaquinone-4 occurs in the fetal liver.

The radioactivity in fetal gut increased with time, which suggests that $[^{14}C]$menaquinone-4 is actively excreted into bile in the fetus on day 19 of gestation, as is the case in male adult rats (7, 19).

In order to determine the transport of menaquinone-4 and its metabolites into milk and blood of sucklings, $[^{14}C]$menaquinone-4 (4 mg/kg) was given orally to lactating rats 14 days after delivery and their sucklings were allowed to suckle after a 2-h abstinence. The concentration of radioactivity in the milk present in a semisolid state in the stomach of sucklings was higher than that in maternal blood, and unchanged menaquinone-4 accounted for approximately 84% of the radioactivity of milk. The milk/blood ratios gradually increased, and were 3.0 at 3 h, 13.8 at 6 h, and 65.1 at 24 h after dosing (Table 2). The decline of radioactivity in the milk was much slower than that in the blood, but at 53 h the milk level had decreased to about 1/3 of the maximum value.

Since these sucklings were not consuming solid food or water during the study, the concentration of radioactivity in the stomach contents of the sucklings may approximate to the actual drug concentration in milk. Comparable results (20) were obtained by measuring the concentration of nifuridide in either milk obtained directly from the mother or milk obtained from the stomach of the nursing sucklings.

It was reported that cow's milk contains a protein complex which is able to bind vitamin K$_1$ in a reversible manner (21). Since menaquinone-4 is relatively easily transferred into milk, it is suggested that a menaquinone-4 binding protein might...
exist in milk. We are planning a study to examine the existence of a binding protein in milk in the near future.

In conclusion, we have found that, in spite of the low placental transfer rate of menaquinone-4, an amount of the unchanged form more than equivalent to the essential amount of vitamin K can be transferred into the fetal liver from the mother, and further, the transfer of maternally administered menaquinone-4 through breast milk to sucklings is quantitatively large in rats. Recently, Hiraike et al. (22) reported that menaquinone-4 administered to the mother was transferred to breast milk in humans. Therefore, our results suggest that prophylactic maternal oral administration of menaquinone-4 may be efficacious for a prophylaxis of neonatal and infantile vitamin K deficiency.

REFERENCES

1) Lane, P. A., and Hathaway, W. E. (1985): Vitamin K in infancy. J. Pediatr., 106, 351–358.
2) Hanawa, Y., Maki, M., Murata, B., Matsuyama, E., Yamamoto, Y., Nagao, T., Yamada, K., Ikeda, I., Terao, T., Mikami, S., Shiraki, K., Komazawa, M., Shirahata, A., Tsuji, Y., Motohara, K., Tsukimoto, I., and Sawada, K. (1988): The second nation-wide survey in Japan of vitamin K deficiency in infancy. Eur. J. Pediatr., 147, 472–477.
3) Deblay, M. F., Vert, P., Andre, M., and Marchal, P. (1982): Transplacental vitamin K prevents hemorrhagic disease of the infants of epileptic mothers. Lancet, I, 1247.
4) Kries, R. von, Shearer, M. J., and Göbel, U. (1988): Vitamin K in infancy. Eur. J. Pediatr., 147, 106–112.
5) Matschiner, J. T., and Doisy, E. A. (1966): Bioassay of vitamin K in chicks. J. Nutr., 90, 97–100.
6) Ullberg, S. (1954): Studies on distribution and fate of 35S-labelled benzylpenicillin in the body. Acta. Radiol. Suppl., 118, 1–110.
7) Hirate, J., Horikoshi, I., Watanabe, J., Tadano, K., Yamato, C., and Fujita, T. (1985): Disposition of menaquinone-4 following intravenous and oral administration to rats. Oyo Yakuri-Pharmacometrics, 29, 775–781.
8) Wiss, O., and Gloor, U. (1966): Absorption, distribution, storage and metabolites of vitamin K and related quinones. Vitam. Horm., 24, 575–586.
9) Abe, K., Hiroshima, O., Ishibashi, K., Ohmae, M., Kawabe, Y., and Katsui, G. (1979): Fluorimetric determination of phylloquinone and menaquinone-4 in biological material using HPLC. Yakugaku Zasshi (in Japanese), 99, 192–200.
10) Sann, L., Leclercq, M., Guillaumont, M., Trouvez, R., Bethenod, M., and Bourgeay-Causse, M. (1985): Serum vitamin K1 concentration after oral vitamin K1 administration in low birth-weight infants. J. Pediatr., 107, 608–611.
11) Shearer, M. J., Barkhan, P., and Webster, G. R. (1970): Absorption and excretion of an oral dose of tritiated vitamin K1 in man. Br. J. Haematol., 18, 297–308.
12) Shearer, M. J., McBurney, A., and Barkhan, P. (1974): Studies on the absorption and metabolism of phylloquinone (vitamin K1) in man. Vitam. Horm., 32, 513–542.
13) Shino, M., Yamashiro, T., Yamada, K., Mori, Y., Sato, C., Kawabe, Y., and Okada, K. (1982): Determination of menaquinone-4 in plasma after administration of menaquinone-4 dosage forms in healthy human subjects. Yakugaku Zasshi (in

J. Nutr. Sci. Vitaminol.
Japanese), 102, 651–658.

14) Shearer, M. J., Barkhan, P., Rahim, S., and Stimmmer, L. (1982): Plasma vitamin K1 in mothers and their newborn babies. *Lancet*, II, 460–463.

15) Hamulyá, K., de Boer-van den Berg, M. A. G., and Thijsen, H. H. W. (1987): The placental transport of [3H]vitamin K1 in rats. *Br. J. Haematol.*, 65, 335–338.

16) Guillaumont, M. J., Durr, F. M., Combet, J. M., Gueho, A. G., Fournier, B. M., Sann, L., Leclercq, M. A., and Frederich, A. H. (1988): Vitamin K1 diffusion across the placental barrier in the gravid female rat. *Dev. Pharmacol. Ther.*, 11, 57–64.

17) Taggart, W. V., and Matschiner, J. T. (1969): Metabolism of menadione-6,7-3H in the rat. *Biochem. J.*, 8, 1141–1146.

18) Haroon, Y., and Hauschka, P. V. (1983): Application of highperformance liquid chromatography to the assay phylloquinone (vitamin K1) in rat liver. *J. Lipid. Res.*, 24, 481–484.

19) Konishi, T., Baba, S., and Sone, H. (1973): Whole-body autoradiographic study of vitamin K distribution in rat. *Chem. Pharm. Bull.*, 21, 220–224.

20) Pohland, R. C., and Byrd, T. K. (1987): A comparison of two methods of milk sampling. *Toxicologist*, 7, 726.

21) Fournier, B., Leclercq, M., Audigier-Petit, C., Letoublon, R., Got, R., and Frot-Coutaz, J. (1987): Vitamin K1 binding protein in milk. *Int. J. Vit. Nutr. Res.*, 57, 145–150.

22) Hiraie, H., Kimura, M., and Itokawa, Y. (1989): Administration of vitamin K2 syrup to mother before delivery. *Vitamins (J. Vitamin Soc., Jpn.)*, 63, 25–28.