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The placental transport of [^3H]vitamin K_1 in rats

KARLY HAMULYÁK, MARIAN A. G. DE BOER-VAN DEN BERG, HENK H. W. THIJSSSEN,*
H. COENRAAD HEMKER AND CEES VERMEER *Departments of Biochemistry and *Pharmacology,*
University of Limburg, Maastricht, The Netherlands

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Summary. In this paper we describe the placental transport of [^3H]vitamin K_1 in pregnant rats during the first 24 h after the oral administration of the vitamin. Vitamin K_1 in the fetal livers ranged from 0.13% (3 h) to 2% (24 h) of the values found in the corresponding maternal livers. In spite of the low placental transfer of vitamin K, we found no accumulation of coagulation factor precursors in the fetal rat liver microsomes as could be expected in vitamin K deficiency. Moreover, we could not demonstrate any difference between adult and fetal

rat liver microsomes with regard to the sensitivity for warfarin. From these results we conclude that a substantial placental barrier exists for the transport of pharmacological amounts of vitamin K_1 but that under physiological conditions sufficient vitamin K_1 appears to be present in the fetal liver to ensure a full carboxylation reaction. The vitamin K-dependent carboxylase activity rate of adult and fetal rat liver microsomes was comparable, indicating that the newborn rat has an adequate carboxylating system.

It has been well established that the concentrations of the fat-soluble vitamins in umbilical cord plasma are lower than those in maternal plasma (Baker *et al*, 1975; Dancis & Schneider, 1975; Shearer *et al*, 1982). One of these vitamins is vitamin K, which is required for the synthesis of the coagulation factors II, VII, IX and X and the anticoagulation factors Protein C and S. Shearer *et al* (1982) demonstrated the vitamin K_1 concentrations in umbilical cord plasma to be much lower than those in maternal plasma. A relatively small increase of vitamin K_1 was observed in the umbilical cord plasma after its administration to the mother, shortly before delivery.

At birth the plasma concentrations of the vitamin K-dependent coagulation factors in full-term normal infants are decreased to levels of 30–60% of normal (Hathaway & Bonnar, 1970). These low levels have been attributed to a partial vitamin K-deficiency, but in that case the presence of descarboxy-coagulation factors (PIVKAs) in the umbilical cord plasma would be expected, and also a substantial difference between the coagulation factor levels in the plasma from newborns, who received vitamin K prophylactically at birth and those who did not. Both points are rather controversial in the literature (Malia *et al*, 1980; van Doorm *et al*, 1977; Muntean *et al*, 1979; Corrigan & Krijie, 1980; Motohara *et al*, 1985). We have studied the placental transport of [^3H]vitamin K_1 in pregnant rats and its distribu-

tion in some maternal and fetal tissues at various times after oral administration of the vitamin. Furthermore we have tried to find evidence for the occurrence of a partial vitamin K-deficiency in the fetal rat livers. The results of these experiments are presented in this paper.

EXPERIMENTAL PROCEDURES

Materials. [^3H]vitamin K_1 (320 Ci/mol), which was a kind gift, as well as non-labelled vitamin K_1 (Konaktion®) were provided by Hoffmann-La Roche (Basle, Switzerland). The synthetic pentapeptide Phe-Leu-Glu-Glu-Leu (FLEEL) was obtained from Vega Biochemical Co. (Tucson, U.S.A.) and warfarin and dithiothreitol from Sigma (Saint Louis, U.S.A.). Protosol, Biofluor and Atomlight were from New England Nuclear (Dreieich, F.R.G.). $\text{NaH}^{14}\text{CO}_3$ (56 Ci/mol) was purchased from Amersham (U.K.). All other chemicals were obtained from Merck (Darmstadt, F.R.G.).

Methods. Pregnant, 12-week-old rats (weights 240–270 g) of the Lewis strain were used throughout our experiments. [^3H]vitamin K_1 (0.04 mCi) was administered orally in 0.5 ml sunflower oil, 24–48 h before the planned delivery. At the indicated times the animals were bled by heart puncture under ether anaesthesia and the blood was collected in heparinized plastic tubes. The various organs as well as the fetuses were removed immediately and washed with icecold buffer A (0.1 M NaCl, 0.05 M Tris-HCl, pH 7.4). Blood samples (100 μl) as well as 200 mg pieces of the maternal and fetal livers and the placentae were digested in Protosol and counted for total radioactivity. [^3H]Vitamin K levels in blood

Correspondence: Dr Karly Hamulyák, Department of Biochemistry and Internal Medicine, University of Limburg, P.O. Box 616, 6200 MD Maastricht, The Netherlands.

and liver tissues were obtained from hexane extracts as described by Knauer *et al* (1976). Reverse-phase thin layer chromatography showed that more than 90% of the hexane extractable radioactivity consisted of vitamin K₁.

Microsomes from adult and fetal livers were prepared as described previously (Vermeer *et al*, 1982). The vitamin K-dependent carboxylation reaction was recorded by incubating the microsomes (1 mg of protein) in 0.25 ml reaction mixtures containing 0.1 M NaCl, 0.05 M Tris-HCl, pH 7.4, 8 mM dithiothreitol, 1 M (NH₄)₂SO₄, 0.2 mM vitamin K hydroquinone, 10 μCi NaH¹⁴CO₃, 12% (v/v) ethyleneglycol, 0.2% (w/v) CHAPS (3-[3-cholamidopropyl]dimethyl ammonio]-1-propane sulfonate) and 4 mM FLEEL. The mixtures were incubated in sealed tubes at 25°C. The reaction was stopped with 2 ml 5% (w/v) trichloroacetic acid at the indicated times and non-bound ¹⁴CO₂ was removed by boiling for 2 min before the samples were counted in Atomlight. The amount of incorporation of ¹⁴CO₂ into endogenous substrate was measured in a similar way, except that the amount of microsomal protein was 2.5 mg and that no FLEEL and (NH₄)₂SO₄ were present in the reaction mixtures. Protein concentrations were measured according to Lowry *et al* (1951).

RESULTS

The appearance and disappearance of vitamin K₁ in the rat blood was measured in a group of three animals after a single oral dose of [³H]vitamin K₁. The radioactivity rapidly appeared in the circulation, reached peak concentrations between 3–4 h (Fig 1). During the first 3–4 h blood radioactivity consisted for more than 90% of vitamin K₁, beyond that time the hexane extractable radioactivity declined more rapidly than the total blood radioactivity, indicating the conversion of vitamin K₁ to more polar components.

Another group of rats was used for the preparation of various organs: the maternal and the fetal livers and the placenta. The total radioactivity as well as the hexane-extractable fractions of these tissues and the maternal blood were measured. The results are given in Table I.

As compared to the maternal liver, only very low amounts of label accumulated in the fetal livers. Moreover it was clear that the main part of the label recovered from the fetal livers originated from polar vitamin K₁-degradation products rather than from vitamin K₁ itself. The total radioactivity found in the placenta appeared to be lower than the amount found in the blood samples of the mother at all time points (20–40%). From these results it is obvious that a considerable placental barrier existed for the transport of vitamin K₁.

Since we were interested in whether the impeded vitamin K transport leads to a partial vitamin K-deficiency in the fetal liver, we investigated the vitamin K-dependent carboxylase system in fetal liver by (a) measuring the levels of endogenous substrates (e.g. carboxylatable precursors of coagulation factors) for carboxylase and (b) measuring the hepatic carboxylase activity. In the case of a vitamin K-deficiency an accumulation of endogenous substrate is to be expected (Vermeer *et al*, 1982). For this purpose the fetal livers were compared with the corresponding maternal livers. No differ-

ences were observed between the fetal and adult rat liver microsomes with regard to the amount of endogenous substrate present. The results are given in dpm/mg protein. In maternal liver 1050 ± 140 (mean ± SD, n = 6) was found, in fetal liver 1130 ± 190 (mean ± SD, n = 6).

The carboxylase activity (dpm/mg protein/min) in maternal liver was 1254 ± 178 (n = 6), in fetal liver 955 ± 106 (n = 5). This indicates that the carboxylating enzyme system in the fetus is comparable with that in adult rats. Since the amounts of endogenous substrate were low in all cases, no evidence could be obtained for a relevant deficiency of vitamin K in the fetal liver. To investigate whether *in utero* fetal carboxylase is as sensitive to warfarin as is maternal carboxylase, we studied the effect of warfarin on the accumulation of endogenous substrate for carboxylase in maternal and fetal liver. Warfarin was administered subcutaneously to pregnant rats, 2 d before the planned delivery as indicated in the experimental procedures.

As is clear from Fig 2, the levels of endogenous substrate were dependent on the intake of warfarin, but at each warfarin dosage they were comparable in maternal and fetal liver.

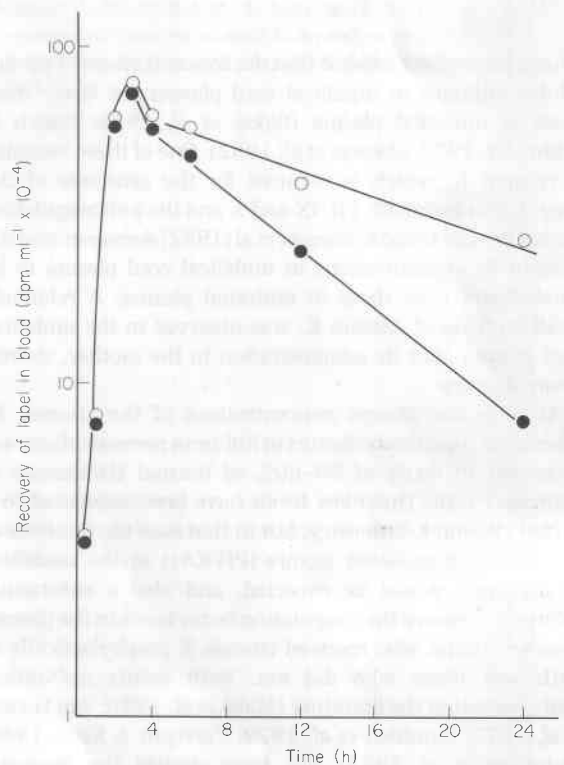


Fig 1. The appearance and disappearance of vitamin K₁ in rat peripheral blood after a single oral dose of [³H]vitamin K₁. The results are given as dpm/ml blood. Total radioactivity is represented by open circles. Hexane extractable radioactivity thereof is given in closed circles.

Table I. The distribution of [³H]vitamin K₁ in maternal and fetal liver. The results are given as dpm/mg protein (means ± SD) for total radioactivity with the hexane extractable radioactivity as a percentage of the total radioactivity. At each indicated time point five animals were studied.

	Time (h) elapsed after start of experiment		
	3	12	24
Maternal liver	28470 ± 3610	14290 ± 4445	18540 ± 11030
Hexane extractable (%)	92 ± 1	96 ± 1	98 ± 1
Fetal liver	160 ± 34	1930 ± 215	2140 ± 760
Hexane extractable (%)	22 ± 2	17 ± 1	17 ± 2
Per cent fetal/maternal liver			
Total radioactivity	0.6	13.5	12
Hexane extractable	0.13	2.2	2

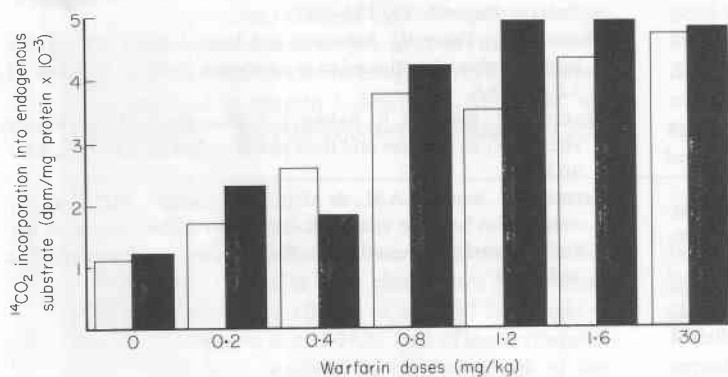


Fig 2. The amount of endogenous substrate for carboxylase in maternal and fetal liver upon the administration of different warfarin doses was measured in microsomal fractions of maternal and fetal liver. Warfarin was administered 2 d before the planned delivery and the animals were killed 24 h after the warfarin treatment. Open columns: maternal liver; closed columns: fetal liver.

DISCUSSION

The first indication for the existence of a placental barrier for vitamin K was reported by Shearer *et al* (1982). Since these authors worked with human volunteers, the actual vitamin K concentrations in the liver could not be determined, however. Using pregnant rats as an experimental animal system, we have demonstrated in this paper that after the oral administration of vitamin K₁ to the mother, the amount of the vitamin (expressed per mg of protein) in the fetal liver is 1–2% of that of the maternal liver. The fetal livers contained relatively high amounts of vitamin K degradation products, indicating that the placental barrier for these compounds is low. Another possible explanation could be a more rapid degradation of vitamin K₁ in the fetal liver.

Since apparently the placental transport of vitamin K is hampered, it has to be expected that the vitamin K level in the fetal liver is low. In the case of a vitamin K deficiency the accumulation of endogenous substrate for carboxylase (coagulation factor precursors) in the hepatic microsomal fraction has to be expected (Vermeer *et al*, 1982). At later stages also

non-carboxylated coagulation factors can be detected in blood plasma. We have been unable to detect increased amounts of carboxylatable proteins in the fetal livers, i.e. in comparison to the maternal liver carboxylatable substrate, the fetal liver gave comparable values. Therefore, no evidence could be obtained for a vitamin K deficiency in fetal liver, assuming that the synthesis of carboxylatable proteins in fetal liver is comparable with that in adults. Moreover, *in vivo* experiments in which pregnant rats were treated for a short time with different doses of warfarin, did not reveal a relatively greater increase in the accumulation of endogenous substrate in the fetal livers, indicating that the newborn enzyme system is comparably sensitive for warfarin as the adult system. It must be concluded, therefore, that in the fetal liver probably sufficient vitamin K is present for a complete carboxylation reaction. Apparently this conclusion is in contradiction to the observed placental barrier for vitamin K₁, but our results may be explained by assuming that the placental transfer of vitamin K is limited. Physiological concentrations of the vitamin may then be transported without difficulty, but during experiments in which pharma-

cological doses of vitamin K₁ are administered to the animals, the placental transport does not increase proportionally. It would thus be interesting to measure directly the levels of endogenous vitamin K in normal maternal and fetal liver, but up till now the determination of physiological concentrations of vitamin K in tissues has proven to be a difficult task. For technical reasons it was also not possible to simulate in this set of experiments the physiological situation obtained via long-term administration of physiological (low) doses of vitamin K.

We conclude that, in spite of the low placental transfer rate of vitamin K₁, no vitamin K deficiency can be demonstrated in newborn rats under normal conditions. Under the assumption that the same holds for human newborns, it may be questioned, if the administration of vitamin K to all normal newborns at birth is indicated.

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