Presence of 25-Hydroxyvitamin D₃ and 1,25-Dihydroxyvitamin D₃  
24-Hydroxylase in Vitamin D Target Cells of Rat Yolk Sac*  

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In the pregnant rat, the yolk sac, which possesses true placental functions, is a vitamin D target organ. We tested its ability to hydroxylate 25-hydroxy- and 1,25-dihydroxyvitamin D₃ (25-OHD₃ and 1,25-(OH)₂D₃). 24,25-Dihydroxy- and 1,24,25-trihydroxyvitamin D₃ were produced by rat yolk sac homogenates incubated with 25-OHD₃ and 1,25-(OH)₂D₃. Rat yolk sac homogenates also formed small amounts of 25,26-dihydroxyvitamin D₃. These newly synthesized metabolites were isolated and identified by Sephadex LH-20 chromatography, high performance liquid chromatography, and periodate cleavage. Yolk sac 25-OHD₃- and 1,25-(OH)₂D₃-24-hydroxylases were present in mitochondria and were of a mixed function oxidase nature. They were detected in the yolk sac as early as day 12 in the embryonic period and until the end of gestation. No hydroxylation occurred in maternal liver, amnion, fetal brain, or skin homogenates. Both 24-hydroxylases were detected in pure isolated rat yolk sac endodermal cells. This may be of physiological importance, since they are the 1,25-(OH)₂D₃ target cells in the yolk sac. Injection of 1,25-(OH)₂[3H]D₃ into rat yolk sac vitelline veins strongly suggested that the yolk sac produced 1,24,25-(OH)₃D₃ in vivo. We conclude that the yolk sac and more precisely its endodermal cells may help to control vitamin D metabolism within the fetoplacental unit.

It is now well established that in vivo, vitamin D₃ undergoes successive hydroxylations (1, 2). In the liver, it is transformed into 25-hydroxyvitamin D₃. In the kidney, the hydroxylation of this compound in position 1 leads to 1,25-dihydroxyvitamin D₃ (3), considered to be the hormonal form of vitamin D₃. In its target organs (intestine, kidney, etc.), it acts as a steroid hormone, by a mechanism which involves the binding of 1,25-(OH)₂D₃ to specific receptors (4). One of the results is de novo synthesis of a vitamin D-dependent calcium binding protein (5).

Both 25-OHD₃ and 1,25-(OH)₂D₃ can be substrates for 24- or 26-hydroxylases. The 24-hydroxylase system produces 24,25-dihydroxyvitamin D₃ and 1,24,25-trihydroxyvitamin D₃. This system is mainly present in kidney mitochondria (6-8) and also in other tissues such as intestine (9), bone (10, 11), and cartilage (12). The role of these 24-hydroxylated vitamin D metabolites is still controversial. Some authors believe them to be inactivated forms of 25-OHD₃ and 1,25-(OH)₂D₃ (1, 9), others think that 24,25-(OH)₂D₃ acts on bone (13-16) and parathyroid gland (17-19). The 26-hydroxylation of 25-OHD₃ and 1,25-(OH)₂D₃ not only occurs in kidney (20, 21), but also in extrarenal sites (22). Although 25,26-(OH)₂D₃ (22) and 1,25,26-(OH)₃D₃ (21) have both been detected in plasma, their role remains obscure.

During pregnancy, vitamin D metabolism is greatly altered (23-31) and it now seems that the fetoplacental unit can participate in regulating its own vitamin D metabolism. Moreover, experiments have recently shown that placenta (32-34) and fetal kidneys (23, 35-37) convert in vitro 25-OHD₃ into more polar metabolites.

In the rat, the visceral yolk sac is involved in the physiological mother to fetus transfer of nutrients and persists until the end of gestation (38). It is in addition the embryonic precursor of the intestine. We recently showed that the rat yolk sac is a vitamin D target organ since it contains both 1,25-(OH)₂D₃ receptors (39) and a vitamin D-dependent calcium binding protein (40, 41). However, little is known about the endocrinological properties of this yolk sac. In the present work, we investigated this fetal organ for its ability to metabolize 25-OHD₃, 24,25-(OH)₂D₃, and 1,25-(OH)₂D₃ into more polar vitamin D metabolites. Another consideration which prompted this study was the fact that the main vitamin D target organs involved in calcium transfers also produce vitamin D metabolites.

**EXPERIMENTAL PROCEDURES**

**Animals and Materials—**Normal pregnant Wistar rats were obtained from Lessieux (France) and fed ad libitum with a normal diet (UAR 103).

Tritiated 25-hydroxy[26,27-3H]vitamin D₃ (24 Ci/mmol), 1,25-dihydroxy[23,24-3H]vitamin D₃ (90 Ci/mmol), and 24,25-dihydroxy[23,24-3H]vitamin D₃ (60 Ci/mmol) were obtained from Amersham (France). Unlabeled 25-OHD₃ was a gift from Roussel Uclaf (France) and 24,25-(OH)₂D₃ and 1,25-(OH)₂D₃ were kindly supplied by Hoffman-LaRoche (Switzerland). 25,26-Dihydroxyvitamin D₃ used as a standard for high performance liquid chromatography, was generously given by Dr. Redel (Paris).

Analytical grade solvents were obtained from Merck (France). Glass-distilled solvents from Burdick & Jackson Laboratories were used for HPLC. Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) was purchased from Sigma.

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The abbreviations used are: 25-OHD₃, 25-hydroxyvitamin D₃; 1,25-(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 24,25-(OH)₂D₃, 24,25-dihydroxyvitamin D₃; 24,25,26-(OH)₃D₃, 24,25,26-trihydroxyvitamin D₃; Tris buffer, 16 mM Tris/acetate buffer (pH 7.4) containing 196 mM saccharose, 24 mM sodium succinate, and 1.8 mM magnesium acetate; HPLC, high performance liquid chromatography.
yolk sac (42). Five fetuses were injected per mother. After this period, the fetuses were allowed to recover for 0.5, 1, or 2 h, when they were again anesthetized and a cesarean section was performed. Blood was collected from the injected fetuses via the axillary vessels and yolk sacs were carefully removed and immediately frozen in liquid nitrogen. All the samples from one mother were pooled and kept at −20 °C until analysis.

In Vitro Studies: Tissue Preparations—Unless otherwise stated, in vitro experiments were performed on tissues obtained from the normal pregnant rats on days 18 and 19 of gestation. Animals were killed by decapitation.

Homogenates—The uteri were rapidly excised and immediately placed in ice cold 0.15 M NaCl. All subsequent operations were performed at 4 °C. Yolk sacs were dissected free of amnion, fetus, and placenta. The tissue was washed several times with ice cold 0.15 M NaCl and then with 16 mM Tris/acetate (pH 7.4) containing 196 mM succarose, 24 mM sodium succinate, and 1.8 mM magnesium acetate (Tris buffer). Yolk sacs were cut into small pieces and homogenized in 5 volumes of Tris buffer by 4 passes in a Potter-Elvehjem homogenizer kept in ice.

Mitochondrial Preparations—Yolk sac homogenates were centrifuged at 4 °C for 10 min at 400 × g. The pellet was discarded and the supernatant was centrifuged at 4 °C for 20 min at 6000 × g. The mitochondrial pellet was collected and resuspended in Tris buffer. The enrichment of the mitochondrial preparation was tested by determining the succinic dehydrogenase activity using the p-iodonitrotetrazolium violet method (43).

Preparations—Homogenates were centrifuged for 30 min at 135,000 × g in a Beckman L7 75 ultracentrifuge. The final high speed supernatant without the fluffy layer was taken as the cytosol. Pure Isolated Endodermal Cells—These cells were obtained from intact yolk sacs by the action of EDTA in phosphate-buffered saline at 37 °C, as described in detail elsewhere (44). Cells were collected by low speed centrifugation, washed, and immediately suspended in Tris buffer (about 2 × 10^6 cells/ml) for incubation with the labeled sterol. The endodermal cells were identified by their appearance under a light microscope, and their calcium binding protein content was detected by immunocytochemistry (44). The protein contents of the preparations were determined by a modified version of Lowry’s method (45).

Incubation with Labeled Sterols—In a typical experiment, 1 ml of the yolk sac homogenates suspended in 16 mM Tris/acetate (pH 7.4) containing 196 mM succarose, 24 mM sodium succinate, and 1.8 mM magnesium acetate was transferred into a 25-ml Teflon-stopped Erlenmeyer flask which was gassed with O2 for 45 s. Ten pmol of the radioactive sterol (0.25 μCi) dissolved in 10 μl of ethanol were then added, yielding a final concentration of 10 nM. The flask was gassed with pure O2 for a further 45 s and stopped. Incubation was generally performed for 1 h at 37 °C in a water bath with rapid shaking. At the end of incubation, aliquots were taken for duplicate determination of protein concentration and radioactivity. Unlabeled 24,25-(OH)2D3, 1,25-(OH)2D3, and 1,24,25-(OH)3D3 were then added in order to minimize losses during extraction and to serve as internal standards during the HPLC separation. After addition of 2 volumes of methanol and 1 of chlorof orm, radioactivity was extracted by shaking the flask overnight at 4 °C. The mixture was then transferred to a separatory funnel. Addition of 2 volumes of methanol and 1 of chloroform was found to adequately resolve a mixture of 25-OHD3, 24,25-(OH)2D3, 25,26-(OH)2D3, and 1,25-(OH)2D3. Fractions corresponding to 30 s were collected and counted for radioactivity.

Radioactivity was measured in an Intertechnique SL 40 liquid scintillation counter using Picofluor (Packard, France). When necessary, results were corrected for quenching by means of an external standard.

For quantification of the newly formed metabolites, both Sephadex LH-20 chromatography and HPLC were used and gave the same results. Consequently, HPLC was chosen for routine experiments. Metabolite production was expressed as the ratio of tritium present in the appropriate region versus the radioactivity recovered from the column. In all cases, more than 90% of radioactivity was recovered from the HPLC column.

Periodate Reaction—The purified metabolites were submitted to periodate cleavage. Fifty μl of a 5% aqueous sodium meta-periodate (or 50 μl of water as a control) was added to the metabolite dissolved in 50 μl of methanol. The reaction was allowed to proceed overnight at room temperature in the presence of nitrogen. The reaction mixture was then dried under vacuum and twice extracted with 200 μl of CHCl3 which was then evaporated under vacuum. Results are expressed as the per cent of radioactivity lost by the NaIO4-treated sample compared to the control. As another test of the periodate action, aliquots of NaIO4-treated and of untreated samples were injected into an HPLC column eluted with the appropriate straight phase solvent system. Radiochromatograms were obtained as described in the HPLC section.

Sucrose Density Gradient Analysis—The binding of the putative 24,25-(OH)2D3 to the rat serum vitamin D binding protein was tested by sucrose density gradient analysis as previously described (30).

RESULTS

Metabolism of 25-OH[3H]D3 by Yolk Sac Homogenates—After incubation of yolk sac homogenates with 10 nM 25-OH[3H]D3, the radioactive lipid extract was analyzed by HPLC and Sephadex LH-20 chromatography, and the radioactivity profile obtained showed that radioactive products were more polar than 25-OH-D3. The radioactive mixture was dried under vacuum and twice extracted with 200 μl of CHCl3 which was then evaporated under vacuum. Results are expressed as the per cent of radioactivity lost by the NaIO4-treated sample compared to the control. As another test of the periodate action, aliquots of NaIO4-treated and of untreated samples were injected into an HPLC column eluted with the appropriate straight phase solvent system. Radiochromatograms were obtained as described in the HPLC section.

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Metabolism of 25-OH[3H]D3 and 1,25-(OH)2D3 in Rat Yolk Sac
were further purified by HPLC in the 90:10 hexane/isopropyl alcohol solvent system. The 24,25-(OH)_{3}D_{3} and 25,26-(OH)_{3}D_{3} HPLC regions were collected and analyzed by HPLC in the normal and reverse phase systems. The purified putative 24,25-(OH)_{2}[3H]D_{3} and 25,26-(OH)_{2}[3H]D_{3} fractions exhibited a major radioactive peak, which co-eluted with authentic standards in both the straight (Fig. 2, C and D) and reverse phase systems (Fig. 2, E and F). However, the nature of the minor radioactive contaminating product shown in evidence during HPLC in the reverse phase system remains unknown.

In order to confirm the presence of a vicinal glycol grouping, the HPLC-purified putative 24,25-(OH)_{2}[3H]D_{3} and 25,26-(OH)_{2}[3H]D_{3} were submitted to periodate oxidation as described under “Experimental Procedures.” This treatment resulted in losses of 83% and 40% of the radioactivity present in the putative 24,25-(OH)_{2}[3H]D_{3} and 25,26-(OH)_{2}[3H]D_{3}, respectively. In addition, the remaining radioactivity was not found to be associated with the corresponding untreated metabolite standard after HPLC in the straight phase system. This is consistent with the presence of hydroxyl groupings in positions 24,25 and 25,26 of the molecules, taking into account that the radioactivity of the starting 25-OH[3H]D_{3} was located on methyls 26 and 27. To further confirm the 24,25-(OH)_{3}D_{3} structure, the ability of the putative 24,25-(OH)_{2}[3H]D_{3} to be bound by serum vitamin D binding protein was tested. This purified radioactive metabolite was incubated for 1.5 h at 4 °C with 1:20 diluted plasma from vitamin D-deficient rats, and the mixture was submitted to sucrose density gradient centrifugation analysis. The radioactivity was found to be associated with a protein sedimenting at 4 S. The 4 S radioactive peak was not detected when incubations were performed in the presence of a 500-fold excess of unlabeled 24,25-(OH)_{3}D_{3} (results not shown). This indicates that the putative 24,25-(OH)_{3}D_{3} formed by the yolk sac was specifically bound with 1:20 diluted plasma from vitamin D-deficient rats, and the remaining radioactivity was not associated with any known vitamin D metabolites. The second radioactive peak was located in the 1,24,25-(OH)_{3}D_{3} region. This metabolite was purified by HPLC in the straight phase system.

The amount of 25,26-(OH)_{2}[3H]D_{3} formed fluctuated from 1% to 10%, even when incubations were performed in the presence of high concentrations of 25-OH[D_{3} (up to 1 μM) in order to restrict the action of the inhibitors present in rat tissues (6). However, we assumed that under the same conditions of incubation and analysis, maternal kidney homogenates produced 1,25-(OH)_{2}[3H]D_{3}.
system with an 85:15 hexane/isopropyl alcohol solvent mixture. Fig. 4 shows that the radioactivity of the HPLC-purified putative 1,24,25-(OH)₃[3H]D₃ fraction co-eluted as a single peak with standard 1,24,25-(OH)₃[3H]D₃ during HPLC in both the straight and reverse phase systems.

When the purified putative 1,24,25-(OH)₃[3H]D₃ was submitted to the periodate reaction, no significant radioactive loss was observed. However, when the periodate-treated fraction was analyzed by HPLC, radioactivity was no longer present in the 1,24,25-(OH)₃D₃ region. These results are consistent with the presence of a vicinal glycol grouping probably in position 24,25 of the molecule, since the tritium atoms of the starting 1,25-(OH)₂[3H]D₃ were located in positions C₂₃ and C₂₄.

All these results strongly suggest that the new metabolite was 1,24,25-(OH)₃D₃.

Inhibition of the 25-OH₃D and 1,25-(OH)₂D₃ 24-Hydroxylase—Yolk sac homogenates were incubated with 10 nM 25-OH[3H]D₃ or 1,25-(OH)₂[3H]D₃ in the presence of increasing amounts of metyrapone. The lipid extracts were then analyzed by HPLC, using the appropriate straight phase systems. The results shown in Fig. 5 indicate that both 24-hydroxylase activities were inhibited by metyrapone. However, the formation of 1,24,25-(OH)₃[3H]D₃ appeared less affected than that of 24,25-(OH)₂[3H]D₃. This suggests that the 24-hydroxylases are of a mixed function oxidase nature.

It is of interest to note that incubation of yolk sac homogenates with 25-(OH)HID₃ in the presence of high concentrations of metyrapone (5 x 10⁻⁵ M) resulted in the total disappearance of the 24,25-(OH)₂[3H]D₃ peak and in the appearance of a new unidentified radioactive metabolite which in the straight phase HPLC system eluted between the 25-OH₃D and 24,25-(OH)₂D₃ regions. Similarly, when incubations were performed with 1,25-(OH)₂[3H]D₃, the unknown radioactive peak X was greatly enhanced while 1,24,25-(OH)₃D₃ was no longer detected (results not shown).

Two other well known inhibitors of cytochrome P-450, SKF 525 A and carbon monoxide, were used to further specify the nature of the 25-OH₃D₂ and 1,25-(OH)₂D₃ 24-hydroxylase system. The addition of SKF 525 A (5 x 10⁻⁴ M) to the incubation medium resulted in a 100% and 75% inhibition of the 25-OH₃D₂ and 1,25-(OH)₂D₃ production by rat yolk sac homogenates. A pool of yolk sacs from 4 mothers was homogenized. One-ml aliquots were incubated with 10 nM 25-OH[3H]D₃, or 1,25-(OH)₂[3H]D₃, for 15 min at 37 °C in the presence of increasing concentrations of metyrapone. The per cent of transformation into 24,25-(OH)₂[3H]D₃ or 1,24,25-(OH)₃[3H]D₃, was determined in duplicate after HPLC in the straight phase systems. Results are expressed as the per cent of control values (in the absence of metyrapone). C1, 24,25-(OH)₂[3H]D₃; C2, 1,24,25-(OH)₃[3H]D₃. These results were confirmed in another set of experiments.

And 1,25-(OH)₂D₃ 24-hydroxylases, respectively. When homogenates were flushed with carbon monoxide prior to incubation, the formation of both 24-hydroxylated metabolites was greatly lowered (up to 70% inhibition). Such decreases were never observed in control experiments in which nitrogen was used instead of carbon monoxide.

In other respects, in the absence of succinate in the incubation medium, there was no formation of 24-hydroxylated metabolites. All these experiments confirmed the cytochrome P-450 mixed function oxidase nature of the yolk sac 24-hydroxylase system.

Mitochondrial Location of the Enzymatic Activities—Yolk sac mitochondrial preparations were incubated in Tris buffer with 10 nM 25-OH[3H]D₃ or 1,25-(OH)₂[3H]D₃. The HPLC radioactivity profiles obtained for the two lipid extracts were identical with those shown for homogenates in Figs. 1 and 3. Conversely, when yolk sac cytosols were incubated under the same conditions, the radioactivity was recovered after HPLC.
as the untransformed starting metabolite. These results show that the enzymatic activities were mainly of mitochondrial origin.

**Metabolism of 25-OH(3H)D₃ and 1,25-(OH)₂(3H)D₃ by Isolated Yolk Sac Endodermal Cells**—Pure isolated yolk sac endodermal cells (2 x 10⁶ cells/ml of Tris buffer) were incubated intact with 10 nM 25-OH(3H)D₃ or 1,25-(OH)(3H)D₃ for 1 h at 37 °C. The lipids were then extracted and analyzed as described for homogenates. In the three different preparations of endodermal cells tested, the presence of 24,25-(OH)₂(3H)D₃ or 1,24,25-(OH)₃(3H)D₃ was always detected by HPLC in the straight phase systems. These results indicate that both 25-OHD₃ and 1,25-(OH)₂D₃ 24-hydroxylases were present inside the endodermal cells of the rat yolk sac.

**Metabolism of 25-OH(3H)D₃ and 1,25-(OH)₂(3H)D₃ by Yolk Sac Homogenates during Gestation**—Yolk sacs were obtained from normal pregnant females on days 12, 15, 18, 20, or 21 of gestation. Rat yolk sac homogenates were incubated with 25-OH(3H)D₃ or 1,25-(OH)(3H)D₃. As shown in Fig. 6, both 24,25-(OH)₂(3H)D₃ and 1,24,25-(OH)₃(3H)D₃ were continuously formed from days 12-21 of gestation. Production of both metabolites markedly decreased from days 12-18, but tended to rise again at the end of gestation. Production of 24,25-(OH)₂(3H)D₃ was always about 4 times that of 25-OH(3H)D₃. In addition, homogenates from 12-day-old embryos formed 24,25-(OH)₂(3H)D₃ and 1,24,25-(OH)₃(3H)D₃.

**Tissue Distribution Studies**—Tissue distribution studies indicated that 25-OHD₃ and 1,25-(OH)₂D₃ were metabolized by rat yolk sac homogenates but not by amnion, the other component of fetal membranes, or by fetal brain, fetal skin, or maternal liver.

**In Vivo 24-Hydroxylation of 1,25-(OH)₂D₃ by Yolk Sac**—To test whether 24-hydroxylation occurred in vivo, we chose to follow the metabolism of 1,25-(OH)₂D₃, since in vitro experiments had indicated that 24-hydroxylation of the latter appeared to be greater than that of 25-OHD₃. Thus, 1,25-(OH)₂(3H)D₃ was injected into the vitelline vein of the yolk sac from normal pregnant rats. Yolk sacs and fetal plasma were collected 0.5, 1, and 2 h later. Their lipid extracts were analyzed for the presence of 1,24,25-(OH)₃(3H)D₃ by HPLC in the straight phase system.

**TABLE 1**

| Time after injection | Yolk sac | Fetal plasma |
|----------------------|----------|--------------|
| 0.5                  | 25,100   | 0.71         |
| 1                    | 20,900   | 3.57         |
| 2                    | 16,700   | 3.21         |

This study clearly demonstrates that rat yolk sac homogenates metabolize 25-OHD₃ to more polar metabolites, i.e. 24,25-(OH)₂D₃ and to a lesser extent 25,26-(OH)₂D₃. This organ also hydroxylates in vitro 1,25-(OH)₂D₃ in position 24, leading to 1,24,25-(OH)₃D₃. The identification of these three metabolites is reasonably certain, since it is based on the results of several biochemical techniques, including Sephadex LH-20 chromatography, HPLC in straight and reverse phase systems, and periodate sensitivities.

**The 25-OH(3H)D₃ and 1,25-(OH)₂D₃ 24-hydroxylase activities of the yolk sac were found to be associated with mitochondria. They were inhibited by metyrapone, SKF 525 A, and carbon monoxide and thus are of a cytochrome P-450 mixed function oxidase nature. These properties are similar to those reported for the 24-hydroxylase in adult kidney (7, 8). Under our experimental conditions, 1,25-(OH)₂D₃ seemed to be a better substrate than 25-OHD₃ for the rat yolk sac 24-hydroxylase enzymes. Such preferential transformation of 1,25-(OH)₂D₃ has been reported for rat intestinal 24-hydroxylase (9).

It was important to test whether the 25-OH(3H)D₃ and 1,25-(OH)₂D₃ 24-hydroxylases are active in vivo. Injection of tritiated 1,25-(OH)₂D₃ into the vitelline vein of the yolk sac demonstrated that 1,25-(OH)₂D₃ 24-hydroxylase may function in vivo. Yolk sac may thus be considered as one of the sources of 1,25-(OH)₂D₃ that we detected in the fetal plasma. The presence of this trihydroxylated vitamin D metabolite has not so far been demonstrated in the fetus. In the adult, 1,24,25-(OH)₃D₃ is often considered as an inactivated form of 1,25-(OH)₂D₃ (1). However, one cannot exclude that it would be active per se in the fetal unit (46). With respect to 24,25-(OH)₂D₃, several workers recently drew attention to this metabolite during fetal life (24, 25, 31). 24,25-(OH)₂D₃ is known to be specifically accumulated in the fetal skeleton and may be important in early bone formation (26). In addition, the plasma levels of this metabolite exhibit a mother to fetus...
gradient (23, 30, 31). However, the origin of 24,25-(OH)\textsubscript{2}D\textsubscript{3} in the rat fetus remains unknown. The present study suggests that yolk sac is a potential source of 24,25-(OH)\textsubscript{2}D\textsubscript{3} for fetus.

This, our findings concerning vitamin D metabolism by the yolk sac, together with those describing its ability to transform progesterone (47), extend the function of the yolk sac in steroid hormone metabolism.

We found that yolk sac homogenates formed 24,25-(OH)\textsubscript{2}D\textsubscript{3} and 1,24,25-(OH)\textsubscript{3}D\textsubscript{3} throughout the second half of gestation. During this period, changes in the in vitro metabolism of 25-OHD\textsubscript{3} and 1,25-(OH)\textsubscript{2}D\textsubscript{3} were observed. This may be of physiological significance, although it should be remembered that several uncontrollable parameters, such as endogenous substrate or inhibitor concentrations, may interfere in the enzymatic determinations. It is worth noting that as early as day 12 of gestation, 25-OHD\textsubscript{3} and 1,25-(OH)\textsubscript{2}D\textsubscript{3} 24-hydroxylases are present in the yolk sac and also in the embryo. This is the first evidence for vitamin D metabolism during embryonic life in the rat.

The presence of 25-OHD\textsubscript{3} and 1,25-(OH)\textsubscript{2}D\textsubscript{3} 24-hydroxylases in the yolk sac makes this vitamin D target organ (39-41) comparable to the main target organs like intestine or bone, which are also extrarenal sites of 24-hydroxylation (9-11). In these organs, the identification of the cells containing the 24-hydroxylases in the yolk sac makes this vitamin D target organ (39-41).

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