Kidney International, Vol. 54 (1998), pp. 1455–1462

Mild vitamin A deficiency leads to inborn nephron deficit in the rat

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Background. Vitamin A plays a critical role in fetal organogenesis, and its severe deficiency during pregnancy is known to result in malformations of several organs, including the kidney. However, the consequences of mild vitamin A deficiency (VAD) has received little attention. In the present study, we examined the effect of *in utero* exposure to mild VAD on renal organogenesis.

Methods. A rat model of mild VAD compatible with normal gestation was developed. Plasma retinol was determined by reverse phase HPLC in mothers and fetuses. Nephron counting was performed in kidneys of fetuses and pups issued from control and VAD mothers. Metanephroi explanted from 14-day-old fetuses from both groups were cultured in the presence or absence of retinoic acid (RA), and growth and differentiation were assessed. c-ret expression was analyzed from fetuses exposed *in utero* to VAD or to normal vitamin A status and also in metanephroi grown in culture with or without RA using RT-PCR.

Results. The 50% reduction in circulating vitamin A levels induced by vitamin A deprivation in pregnant rats did not affect the overall fetal development. However, the number of nephrons was reduced by 20% in 21-day-old VAD fetuses. The number of nephrons was closely correlated with circulating vitamin A level in both VAD and control fetuses. Metanephroi taken from VAD fetuses developed to a lesser extent *in vitro*, but their capacity to respond to exogenous retinoic acid was not altered. Finally, we found that the expression of the proto-oncogene c-ret was modulated according to the retinoid environment.

Conclusion. We conclude that vitamin A supply to the fetus is critical in determining the number of nephrons. Data available thus far on the frequency of mild VAD during pregnancy and on the long-term consequences of inborn nephron deficit highlight the clinical relevance of the present study.

Vitamin A deficiency (VAD) is a massive public health problem, mostly in developing countries where it is often part of complex nutritional deficiencies [1–3]. An insufficient vitamin A supply for basic needs may also be related to dietary habits, whatever the caloric intake, and to noncontrolled weight-reducing diets [1]. Finally, low circulating levels of retinol and RBP have been reported in patients with liver disease, gastrointestinal disorders or chronic alcoholism [4–6]. Whatever the origin of vitamin A deficiency, women of child-bearing age are particularly at risk because of the extra requirements of this nutrient during pregnancy and lactation [7, 8]. Due to the essential role of vitamin A and its metabolites during prenatal development, vitamin A status is critical for the fetus [9–12]. This is also the time point in life during that a deficit is the most likely to occur, since in addition to a possible vitamin A deficiency in the mother, any reduction in the placental blood flow, irrespective of the mother's status, will reduce the supply of vitamin A to the fetus. It has been recognized for decades that severe maternal vitamin A deficiency results in fetal death or major congenital malformations in the offspring [13, 14]. By contrast, the consequences of mild vitamin A deficiency during pregnancy have received little attention because the resulting newborns are apparently normal [8, 15]. Nevertheless, a mild vitamin A deficiency may cause clinically silent defects in organogenesis that are not recognized at birth, but may induce long-term functional consequences. Since vitamin A and retinoic acid have recently been reported to be key determinants in the control of nephron number in rat metanephric organ cultures [16], the kidney appears as one of the organs whose development might be impaired by an insufficient vitamin A supply.

In the current study we examined the renal development in a rat model of *in utero* exposure to a mild vitamin A deficiency, allowing an overall normal development to occur. We found that a reduction of circulating vitamin A in the mother induced a nephron deficit in the fetus. The number of nephrons was strictly correlated to circulating levels of vitamin A. We also found that expression of the protooncogene c-ret, which was recently reported to play

Key words: vitamin A in pregnancy, retinoic acid, metanephros, nephron number, c-ret proto-oncogene.

Received for publication February 24, 1998
and in revised form June 2, 1998
Accepted for publication June 2, 1998

an essential role in renal organogenesis [17], was modulated by retinoid environment, thus indicating that the control of nephron mass by vitamin A might be partly mediated by the tyrosine kinase receptor Ret. The clinical implication of these studies is highlighted by recent observations that inborn nephron deficit, even a moderate deficit, represents a potential risk factor for the progression in patients with chronic renal disease [18–20]. Furthermore, some have also suggested that a reduced number of nephrons favors the development of hypertension [18].

METHODS

Animals

Female Sprague-Dawley rats aged 30 days (weight around 100 g) were assigned to two groups. The control group received the standard chow for pregnant and lactating rats (19,800 IU/kg vitamin A). Animals of the vitamin A-deficient group (VAD) were fed the same diet with no vitamin A (100 ± 100 IU/kg). Both diets were purchased from UAR Laboratory (Villemoison sur Orge, France). The females were maintained on their respective diets for three weeks before mating. They were then housed with males overnight and those having vaginal smears containing spermatozoids the following day were housed separately; this day was considered to be day 0 of gestation. The two diets were maintained until the day of experiment. Body weight was recorded three times a week.

Experimental design

Six pregnant females of the VAD group and five from the control group were used on day 21 of gestation. They were weighed and anesthetized with sodium pentobarbital (5 mg/100 g body wt), and the fetuses were delivered by caesarian section. Four fetuses were randomly taken, weighed, and their blood collected from axillary vessels for plasma retinol assay. The left kidney was removed, weighed and prepared for nephron counting. Blood samples were taken from the mothers before sacrifice for plasma retinol determination. In three other females of each group, fetuses were used on day 15 of gestation and metanephroi collected for analysis of c-ret expression. Eight pregnant females of each group were allowed to deliver spontaneously, and the pups weighed four hours later. The litters were reduced to eight pups taken at random. All the pups were weighed on postnatal days 7 and 14. Four pups were randomly taken from each litter on day 14 and used for plasma retinol determination and nephron counting. Two additional pregnant females of the control group were given a subcutaneous injection of 20 mg/kg of retinoic acid in peanut oil, on day 11 of gestation. The number of nephrons was determined in four 14-day-old pups of each litter and compared to the nephron number present in pups born to two other control mothers injected with peanut oil.

Control and VAD females were also used for *in vitro* experiments using metanephric organ culture. Six pregnant females of each group were anesthetized on day 14 of gestation, fetuses were aseptically removed and metanephroi collected for culture. After six days of culture in the presence or absence of retinoic acid, they were prepared either for glomerular labeling and protein assay, or for analysis of c-ret expression.

Metanephric organ culture

Metanephric organ culture was performed as previously described [21, 22]. Briefly, the metanephroi explanted from 14-day-old fetuses were placed onto a 0.8 μm polycarbonate filter (Millipore, Saint-Quentin-en-Yvelines, France), floating on a defined serum-free medium and incubated for six days in 35 mm Petri dishes at 37 ± 0.5°C in a humidified incubator with 5% CO_2. Defined medium was DMEM/Ham's F12 (vol/vol) supplemented with HEPES (15 mM), sodium bicarbonate (45 mM, pH 7.45), transferrin (6.2×10^{-8} M), selenium (6.8×10^{-9} M), insulin (8.3×10^{-7} M), triiodothyronine (2×10^{-9} M) and prostaglandin E1 (7×10^{-8} M). All reagents were purchased from Sigma (Saint-Quentin-Fallavier, France). This medium is well suited for metanephros differentiation [22].

A stock solution of *all-trans*-retinoic acid 10 mM was prepared in absolute ethanol and stored at −20°C in the dark. It was used in the culture medium at a 100 nanomolar concentration. For each fetus, one kidney was grown in control medium, and the other in the retinoic acid supplemented medium.

Nephron mass determination in the entire kidney

The number of nephrons was determined in kidneys of 21-day-old fetuses and 14-day-old pups by the method of Damadian, Shawayri and Bricker [23] adapted for immature kidneys [24]. Whole kidneys were incubated in 50% hydrochloric acid for 10 to 50 minutes at 37°C, the incubation time being dependent on the kidney weight. Kidneys were rinsed with tap water and stored overnight at 4°C in a gauged flask. Following mechanical dissociation, tubules and glomeruli were suspended in water. Three aliquots of known volume were used for counting the glomeruli by three investigators unaware of the specimen origin.

Nephron counting and growth assessment in metanephric organ culture

Nephrons formed *in vitro* can be counted after labeling of all the glomeruli present in the explanted metanephroi after six days of culture, using specific lectin-binding sites located on the external membranes of the podocytes as described previously [25]. Briefly, after fixation with 2% paraformaldehyde in PBS, the explanted metanephroi were detached from the filter, permeabilized with saponin, and labeled with rhodamin-coupled peanut agglutinin. Counting was performed independently by two investigators using

an Optiphot microscope (Nikon, Champigny, France). After counting, growth of the explanted metanephroi was determined by their protein content. The labeled metanephroi were rinsed in distilled water and sonicated for 15 seconds in individual tubes containing 0.5 ml of distilled water. Protein content was measured in duplicate, according to Lowry et al's procedure [26] modified by Larson, Howlett and Jagendorf [27], using bovine serum albumin as standard.

Reverse-transcriptase polymerase chain reaction analysis

c-ret expression was analyzed in metanephroi from 15-day-old embryos exposed *in utero* to VAD or to normal vitamin A status, and also in metanephroi taken from 14-day-old control embryos and grown for six days under paired conditions, that is, in the presence or absence of retinoic acid (100 nM). Messenger RNAs were extracted and pooled from at least eight metanephroi, using Dynabeads mRNA purification kit (Dynal, Oslo, Norway). Following reverse-transcription according to Kinoshita et al [28], amplification for c-ret was performed based on the nucleotide sequence Genbank X67812, using the following primers: (S), 5′-GCGCCCCGAGTG TGAGGAATGTGG-3′, and (AS), 5′GCTGATGCAATGGGCGGCTTGTGC-3′, leading to a PCR product size of 442 bp. Primers for β-actin were used as an internal control to normalize for c-ret expression and were: (S), 5′-AAGAGAGGCATCCTGACCCT-3′, and (AS), 5′-GGCCATCTCTTGCTCGAAGT-3′, with a predicted product size of 504 bp. The reaction mixture was subjected to 30 cycles of amplification in a thermal cycler (Appligene Oncor, Illkirch, France) using 200 μM of each dNTP and 0.75 U Pro-HA DNA polymerase (Eurogentec, Seraing, Belgium). Each cycle consisted of a heat-denaturation step at 95°C for one minute, annealing of primers at 63°C for one minute, and polymerization at 72°C for one minute. To perform relative quantification of gene expression as proposed by Kinoshita et al [28], c-ret and β-actin mRNAs were amplified as follows: first, primers for c-ret mRNA sequence were added to the reaction mixture; then after 5 cycles of amplification, primers for β-actin were added for 25 additional cycles. The PCR products were visualized by UV transillumination and photographed using 667 Polaroid films. Bands densitometry was performed using image analysis software (NIH Image).

Plasma retinol determination

Plasma vitamin A concentration was determined by reversed phase HPLC [29]. Briefly, samples were mixed with an ethanol-solution. Two milliliters of n-hexane/butylated hydroxytoluene were added for vitamin A extraction, and the n-hexane phase was removed by evaporation under nitrogen. The residue was dissolved in 200 μl of the mobile phase methanol/dichloromethane 65/35, and 150 μl were injected into a HPLC pump (Waters, Saint-Quentin en Yvelines, France) linked to a multiwave length detector. Detection was performed at 325 nm at a flow rate of 2 ml/min. Separation was performed on a nucleosil C-18 column with a precolumn module (Life Science International, Cergy Pontoise, France). Retinol was used as external standard and retinol laurate as internal standard. All reagents were of ultrapure grade (Sigma, Saint-Quentin-Fallavier, France).

Statistics

All values are expressed as means ± SEM. Control and VAD data were compared by Mann-Whitney *U*-test. The Wilcoxon's test was used for comparison of paired *in vitro* data. Significance was determined by $P < 0.05$. The relationship between two parameters was calculated by determining Pearson's correlation coefficient.

RESULTS

In vivo experiments

The body growth of VAD and control females before mating were similar (112 ± 5 g weight gain vs. 109 ± 8 g, $N = 5$ and 6, respectively). Fertility rates were 88 and 87.5%, respectively. Maternal growth during pregnancy was regular in both groups, resulting in a similar weight gain (172 ± 10 g, $N = 5$, vs. 170 ± 15 g, $N = 6$, respectively). Plasma retinol concentration measured on day 21 of gestation was decreased by approximately 50% in VAD females (14.6 ± 1.1 μg/dl, $N = 5$) as compared to controls (30.5 ± 2.8 μg/dl, $N = 6$, $P < 0.001$).

The same number of fetuses was found in VAD and control mothers (11.9 ± 0.6 and 12.8 ± 0.7, respectively). No obvious developmental defect was observed in any of the 21-day-old fetuses from either group. Plasma retinol concentration in the fetus correlated to that of the mother (Fig. 1). As reported in Table 1, plasma retinol concentration in the VAD fetuses was reduced by about 50% on average. There was no delay in body or kidney growth. However, the mean number of nephrons was reduced by 20%. When VAD and control data were plotted together, the number of nephrons significantly correlated to plasma retinol (Fig. 2). It also correlated for each group taken separately ($r = 0.54$, $N = 20$, $P < 0.05$, for the VAD group; $r = 0.51$, $N = 24$, $P < 0.01$, for controls). In VAD females allowed to deliver spontaneously, the duration of pregnancy was normal. As shown in Table 2, the mean birth weight of pups born to VAD mothers did not differ significantly from that of pups born to control mothers. The same held true for body wt on day 7 after birth, that is three to four days after nephron induction was completed [30]. The final number of nephrons counted in 14-day-old pups was reduced by 20%, that is, in the same proportion as the number counted in 21-day-old fetuses. Pups, already deficient in vitamin A at birth and breast fed by mothers maintained on a VAD diet, had a very low plasma retinol

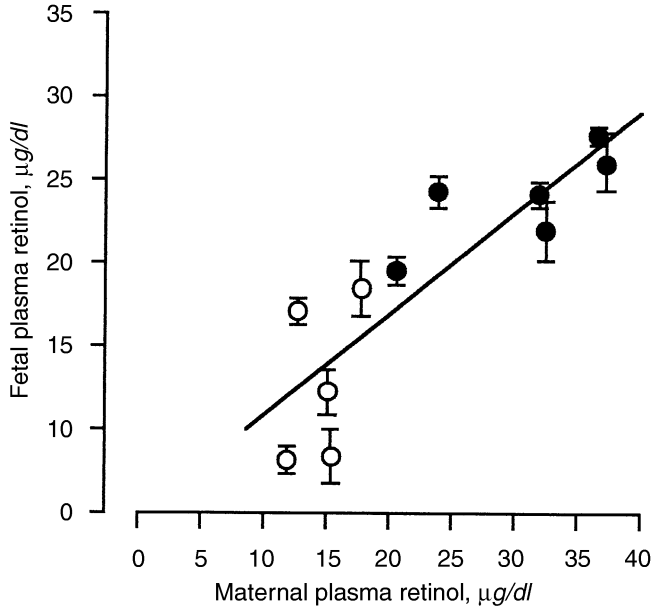

Fig. 1. Fetal plasma retinol as a function of maternal plasma retinol at 21 days of gestation in control (●) and vitamin A deficiency (VAD; ○) groups. Means ± SEM per litter: $r = 0.862$, $P < 0.001$.

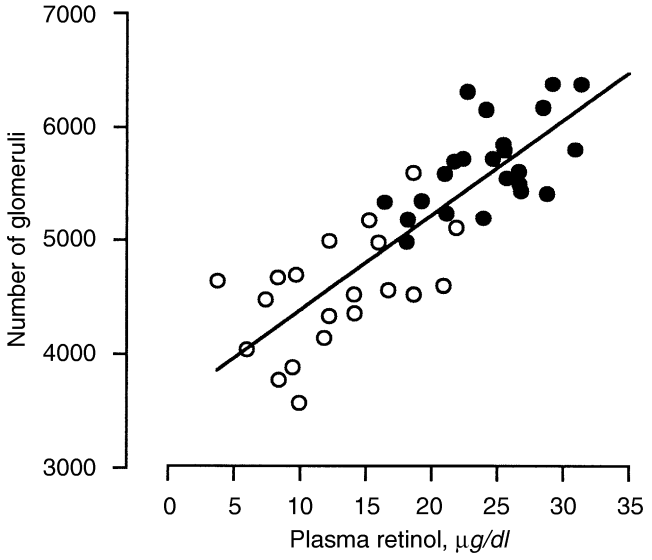

Fig. 2. Number of glomeruli as a function of fetal plasma retinol in 21-day-old fetuses in control (●) and vitamin A deficiency (VAD; ○) groups: $r = 0.829$, $N = 44$, $P < 0.001$.

Table 1. Plasma retinol, body and kidney weights and number of glomeruli in 21-day-old fetuses issued from control and vitamin A deficient (VAD) mothers

Groups	Plasma retinol *μg/dl*	Body weight *g*	Kidney weight *mg*	Number of glomeruli per kidney
Control	24.0	5.27	26.0	5,621
$N = 24$	± 0.8	± 0.09	± 0.9	± 86
VAD	12.9[a]	5.14	27.9	4,511[a]
$N = 20$	± 1.2	± 0.08	± 0.5	± 124

Values are means ± SEM.
[a] $P < 0.001$ vs control

Table 2. Body weights at birth, at 7 days and at 14 days, and kidney weights and number of glomeruli in 14-day-old fetuses issued from control and vitamin A deficient (VAD) mothers

Groups	Body weight *g*			14 day kidney weight *mg*	14 day number of glomeruli
	Birth	7 days	14 days		
Control	5.77	16.1	30.2	198	34,249
$N = 32$	± 0.08	± 0.7	± 0.4	± 5	± 407
VAD	6.02	14.7	22.7[a]	146[a]	27,338[a]
$N = 32$	± 0.05	± 1.0	± 0.4	± 4	± 630

Values are means ± SEM.
[a] $P < 0.001$ vs. control

Table 3. Body weights at birth and at 14 days, kidney weights and number of glomeruli in 14-day-old pups born to control and to mothers injected with retinoic acid (RA) on day 11 of gestation

Groups	Body weight *g*		14 day kidney weight *mg*	14 day number of glomeruli
	Birth	14 days		
Control	5.93	31.4	207	35,911
$N = 8$	± 0.08	± 2.5	± 18	± 1,935
RA	6.05	21.2[a]	164[a]	43,322[a]
$N = 8$	± 0.13	± 1.6	± 6	± 1,837

Values are means ± SEM.
[a] $P < 0.001$ vs. control

concentration (4.2 ± 0.8 μg/dl) compared with pups of the control group (26.0 ± 1.9 μg/dl). As expected, they, had reduced body and kidney weights.

Pups born to mothers injected with retinoic acid on day 11 of gestation appeared normal at birth. As shown in Table 3, the mean birth weight was similar to that of control pups. Body and kidney weights on postnatal day 14 were lower. However, the mean number of nephrons was increased by 21% compared to controls.

In vitro experiments

At the time of fetal kidney collection (embryonic day 14), the maternal plasma retinol concentration was about 50% lower in VAD females (12.2 ± 2.2 μg/dl, $N = 6$) than in control females (26.0 ± 0.6; $N = 6$, $P < 0.001$). The mean body wt of embryos issued from both groups did not differ. Metanephroi explanted from VAD embryos and cultured *in vitro* for six days are shown on the Figure 3 and the quantification of growth and differentiation parameters on Figure 4. Despite identical culture conditions, the mean protein content of metanephroi was decreased by 40%, and the mean glomerular number by 30%. It is worthwhile to note that the lower the maternal plasma retinol concentration, the fewer the nephrons formed *in vitro* ($r = 0.883$, $N = 8$, $P < 0.001$). In the presence of 100 nM of retinoic acid in the culture medium, the metanephric growth and differentiation were markedly stimulated in both groups, but proportionally more for the metanephroi explanted from

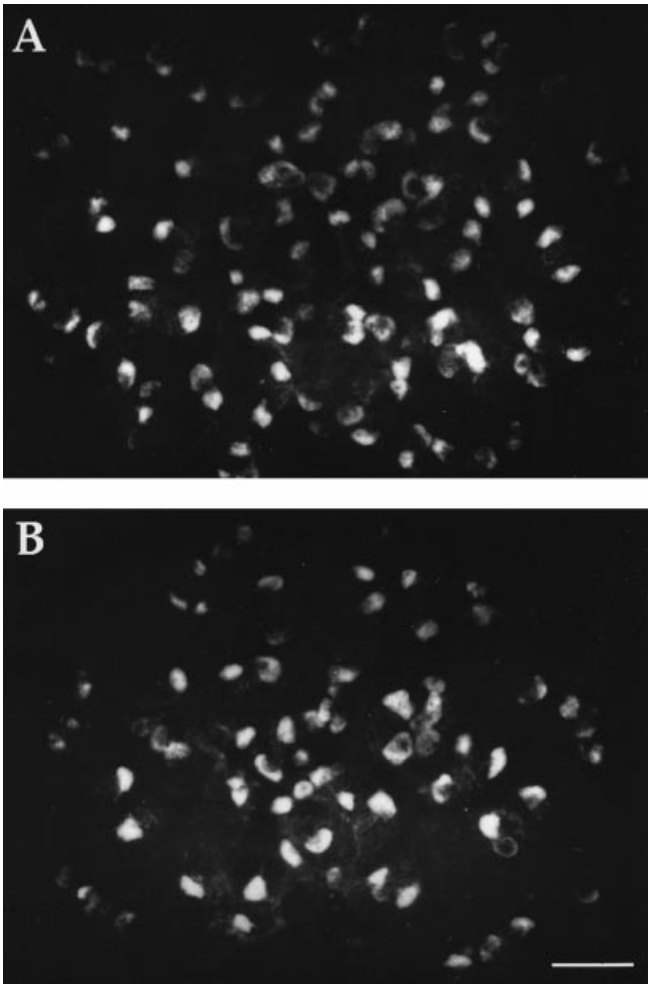

Fig. 3. Metanephros development *in vitro* assessed by lectin histochemistry. Explanted metanephroi from E14 embryos from control (*A*) or from vitamin A deficiency (VAD; *B*) groups were grown for six days in a defined medium. Bar represents 200 μm.

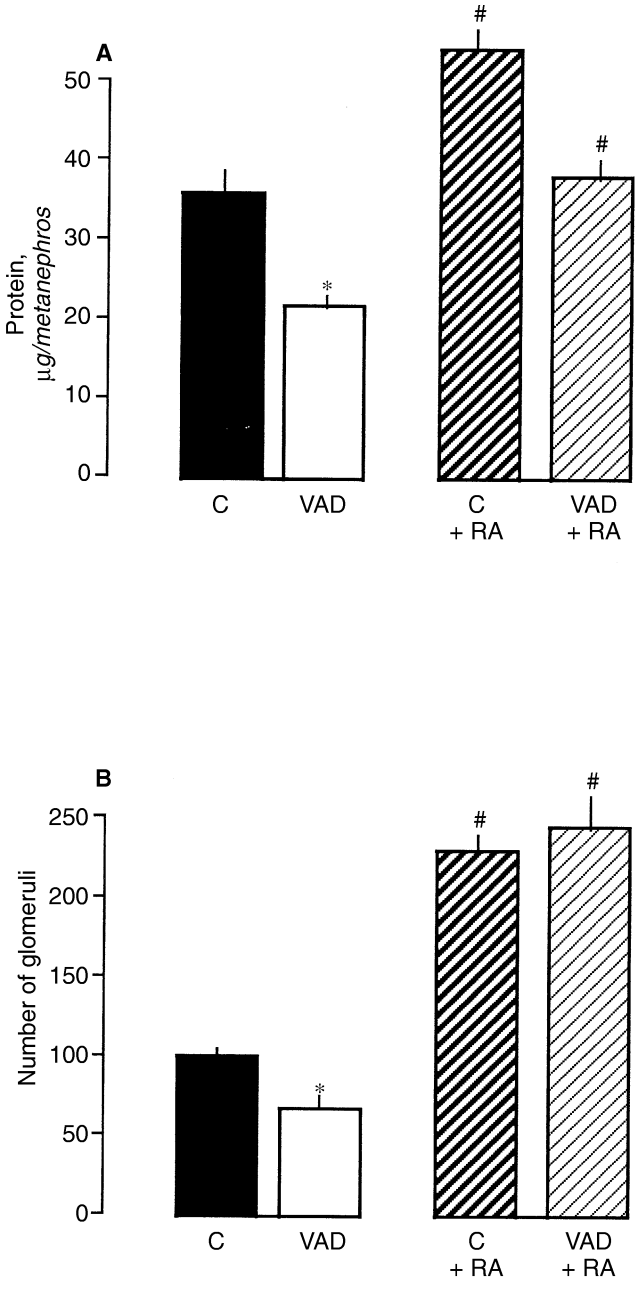

Fig. 4. *In vitro* development of control and vitamin A deficient (VAD) metanephroi. Explanted metanephroi from embryonic day 14 (E14) embryos from control or VAD females were grown for six days in a defined medium in the presence or absence of 100 nM retinoic acid (RA). (*A*) Growth was assayed by total protein content determination. (*B*) Differentiation was analyzed by counting the total number of glomeruli present within the metanephroi. $^{\#}P < 0.001$ as compared to paired controlateral metanephros. $^{*}P < 0.001$, as compared to the control group.

VAD embryos than for those explanted from control embryos (79 ± 9 vs. 42 ± 10% for protein content, and 361 ± 31 vs. 227 ± 15% for glomerular number).

c-ret mRNA expression

As shown in Figure 5A, the c-ret mRNA level was decreased by about 30% in metanephroi from 15-day-old embryos from VAD females as compared to controls. c-ret expression in *in vitro* experiments from 14-day-old embryonic rats is depicted in Figure 5B. The presence of retinoic acid 100 nM in the culture medium for six days induced a sevenfold increase in c-ret mRNAs.

DISCUSSION

The present study demonstrates for the first time that mild vitamin A deficiency during gestation alters nephrogenesis and results in a permanent nephron deficit. Prior to this study, only severe vitamin A deficiencies or excess of vitamin A have been considered as a risk factor for the fetus. The present study also demonstrates that nephron number in the fetus is linearly correlated with circulating vitamin A levels. The correlation was found even for animals in the control group.

In previous animal studies, severe vitamin A deficiency during gestation resulted in multiple malformations in the

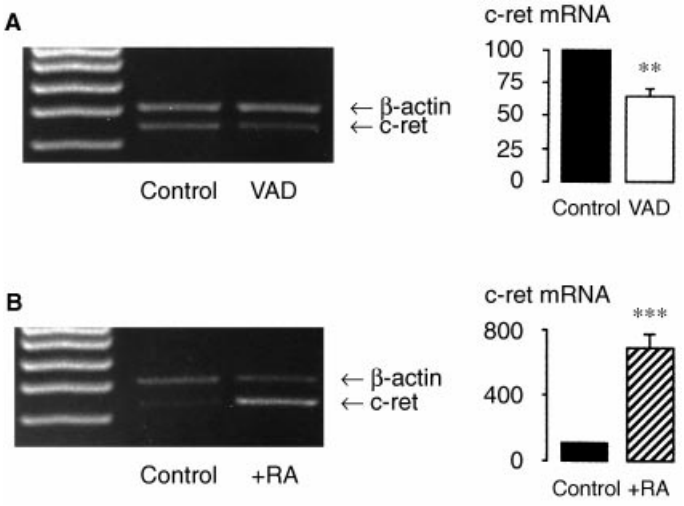

Fig. 5. Product analysis of RT-PCR for c-ret mRNA. (*A*) Metanephroi from 15-day-old embryos from control and VAD females. (*B*) Pairs of metanephroi from 14-day-old embryos from control females cultured for six days in the presence or absence of 100 nM retinoic acid. Two percent agarose gel stained with ethidium bromide revealed the c-ret (442 bp) and the β actin control (504 bp) bands. Blots are representative of experiments repeated five times (A) and three times (B). The amount of mRNA in the different experimental groups was quantified by scanning densitometry and expressed as percent of control.

fetus, with fetal growth retardation or death [13, 14]. In these studies, the animals had been placed for two or three months before mating, onto a diet containing enough carotene for growth but not for appreciable storage of vitamin A. They were then transferred onto a diet completely free of carotene and vitamin A, which was maintained throughout pregnancy. Since hepatic stores of vitamin A take seven to eight weeks to be entirely consumed [31], circulating vitamin A in the mother and in the fetus, although not determined, was likely to be undetectable within a few days, thus explaining the severe developmental defects that were observed. Our goal, by contrast, was to achieve a moderate decrease in circulating vitamin A to determine whether it would influence renal development without leading to either prenatal fatalities or global growth retardation. We therefore deprived female rats from vitamin A for a total of only six weeks, including the duration of gestation, in order to avoid hepatic store exhaustion. This led to about a two times reduction of plasma vitamin A at term, and even two weeks after birth, plasma vitamin A levels, although very low, were still measurable in pups born to VAD mothers.

Vitamin A is transported from the mother to the fetus bound to retinol binding protein [32]. In the present study, fetal plasma retinol variations followed maternal plasma retinol variations. However, the mean plasma retinol concentration in the fetus was about 15% lower than that in the mother. Of interest is the fact that the difference between maternal and fetal plasma retinol was reported to be higher, around 50%, during normal human pregnancy. This may partly explain why the frequency of inadequate vitamin A levels was found to be higher in fetuses than in their mothers [15, 33].

Pups born to VAD mothers had a reduced number of nephrons. This number is clearly inappropriate for a birth body weight as compared to controls. In pups maintained with VAD mothers during lactation, the body weight was, as expected, found to be reduced on day 14, leading to a number of nephrons per body weight ratio similar to that of control pups. However, as observed in littermates weaned on standard diet, total catch-up of growth retardation occurred within two weeks (body wt on day 14 after birth, 30.1 $\pm$ 0.9 vs. 29.7 $\pm$ 1.3 g in control and VAD rats respectively, $N = 32$ in each group). These pups will therefore have an inappropriate number of nephrons for their body weight.

A permanent nephron deficit has been found in humans and animals with intra-uterine growth retardation [34–37]. In the present study, however, pups born to VAD mothers had impaired nephrogenesis, but normal birth weights. Fetal growth retardation is often related to placental blood flow disturbances. We therefore suggest that nephron deficit of growth retardation results from a low vitamin A supply to the fetus. It has indeed been reported that vitamin A and retinol binding protein plasma concentrations, and liver stores of vitamin A are low in growth retarded newborn infants [38, 39].

The complete spectrum of congenital abnormalities in severe vitamin A deficiency is also present in double mutant mice for specific combinations of retinoic acid nuclear receptors [40, 41]. Thus, the developmental defects following severe vitamin A deficiency is likely to result from reduced retinoic acid content. Retinoic acid may also be involved in renal phenotype of mild vitamin A deficiency, since we found that a single injection of retinoic acid to the mother was able to increase nephrogenesis in the fetus. It is also suggested that the content of endogenous retinoic acid was diminished in the metanephroi of vitamin A deficient fetuses that developed *in vitro* to a lower extent. However, the capacity of these metanephroi to respond to exogenous retinoic acid was not altered. These data are consistent with our previous data reporting that retinoic acid controlled the number of nephrons formed in metanephric organ culture in a dose dependent manner [16].

Nephron formation in the metanephric mesenchyme is induced by the tips of the growing branches of the ureteric bud [42]. As a consequence, nephron number depends on the branching capacity of the ureteric bud. We previously reported that retinoic acid stimulates *in vitro* nephrogenesis through an effect on the ureteric bud branching morphogenesis [16]. Consistently, nephron deficits were found in the present study to be proportionally the same in 21-day-old fetuses and in 14-day-old pups, which can be explained by the fact that the ureteric bud no longer branches during late nephrogenesis [43]. No additional deficit could thus be

induced by vitamin A deficiency. We therefore propose that retinoic acid controls branching morphogenesis of the ureteric bud *in vivo* as it does *in vitro*.

From a molecular point of view, one likely candidate to mediate the vitamin A-dependent renal organogenesis is the ret protooncogene, as already proposed [16]. It has been reported that its product, a receptor tyrosine kinase, is expressed in the metanephros and is restricted to the tips of the ureteric bud [44]. The role of c-ret in kidney formation is crucial, since null mice for this gene exhibit renal agenesis or have rudimentary kidneys due to failure of the ureteric bud to develop [17]. In the present study we found that c-ret expression was modulated according to the retinoid environment. This is consistent with previous report showing that c-ret expression can be induced during neuronal differentiation upon retinoic acid exposure [45]. Thus far, no retinoic acid response element has been found within the c-ret promoter [46]. However, retinoic acid nuclear receptors (RAR) are likely to control the c-ret expression that we observed, since renal agenesis or hypoplasia were found in both c-ret null mice and RAR double mutant mice [17, 40].

Finally, our results demonstrate that fetal nephron number strictly correlates to the vitamin A circulating level. This highlights the fact that nephron number is highly dependent on both the placental blood flow and the vitamin A status of the mother. This finding may explain the large variation in the number of nephrons found in otherwise healthy human populations [36, 47–49]. Due to alimentary habits, inadequate intakes of vitamin A may be a common feature, even in developed countries, and in some women the resulting low vitamin A stores may not be sufficient to meet increased demands during pregnancy [7, 8, 15, 50, 51]. A relatively large population of apparently normal newborns may therefore have had altered nephrogenesis, leading to permanent nephron deficit with possible long-term clinical consequences.

ACKNOWLEDGMENTS

This work was presented as oral communication at the 30th Annual Meeting of the American Society of Nephrology, San Antonio, Texas in 1997 and at the European Society of Pediatric Nephrology, Athens, 1997. The authors are very grateful to Drs. L. and G. Striker for critical reading of the manuscript. We also thank the laboratory of "Physiologie et physiopathologie de la nutrition" (URA CNRS 307, Université Denis-Diderot, Paris, France) for utilization of the HPLC apparatus.

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