*Reprod. Nutr. Dévelop.*, 1981, **21** (6A), 961-968.

# Fetal growth and 1,25-dihydroxyvitamin $D_3$ injections into thyroparathyroidectomized pregnant rats

par J.-M. GAREL, M. GILBERT *, P. BESNARD

*Physiologie du Développement, Université Pierre-et-Marie-Curie, 9, quai Saint-Bernard, 75230 Paris cedex 05.*

* *Laboratoire de Physiologie du Développement du Collège de France, place M.-Berthelot, 75231 Paris cedex 05, France.*

**Summary.** Thyroparathyroidectomy (TPTX) in pregnant rats on day 12.5 of gestation was associated with a progressive reduction of dietary intake between days 18 and 21 of gestation. In control mothers with a similar dietary restriction, the fetal plasma calcium and phosphate levels were unchanged, and a slight decrease in fetal weight ($\sim$ 0.8 g) was observed at term. Maternal hypocalcemia in TPTX animals induced chronic fetal hypocalcemia beginning at day 18.5 of gestation ; fetal hyperphosphatemia was only statistically significant on the last day of gestation. Weight, blood glucose and liver glycogen stores, which were greatly decreased in fetuses from untreated TPTX mothers, increased after injection of 1,25-$(OH)_2D_3$ into TPTX mothers. A marked increase in fetal weight ($+$ 1.6-2.0 g) occurred at term with doses ranging from 0.05 to 0.25 $\mu$g/kg of body weight ; higher doses ($\geqslant$ 0.5 $\mu$g/kg) inhibited this improvement. Fetal blood glucose was normalized ($\sim$ 45-50 mg/100 ml) when TPTX mothers received 0.05 to 0.5 $\mu$g/kg, but decreased with higher doses. The highest fetal liver glycogen store (80 mg/g) was achieved using 0.05 $\mu$g of 1,25-$(OH)_2D_3$/kg, this increment being progressively inhibited when larger doses were given. Subcutaneous calcifications were observed in the fetuses of some litters after treatment of the TPTX mothers with 1 $\mu$g of 1,25-$(OH)_2D_3$/kg.

## Introduction.

Since it is known that rat fetuses from thyroparathyroidectomized (TPTX) mothers are characterized by reduced weight (Garel and Geloso-Meyer, 1971) and lower levels of plasma calcium, blood glucose and liver glycogen stores, whereas plasma phosphate levels increase (Porterfield, Whittle and Hendrich, 1975 ; Garel and Gilbert, 1978), and that administration of 1,25-dihydroxyvitamin $D_3$ (1,25-$(OH)_2D_3$) to these mothers recovers the values (Garel, Gilbert and Besnard, 1979 ; Gilbert, Besnard and Garel, 1980), we wished to determine the efficiency of different doses of 1,25-$(OH)_2D_3$ in TPTX mothers.

## Material and methods.

*Experimental procedures.* — Pregnant female rats of the Wistar strain were fed a commercial diet (UAR 103) and water *ad libitum*. The diet contained 0.92 p. 100 calcium, 0.92 p. 100 phosphorus and 0.15 p. 100 magnesium ; its vitamin D content was 4 000 IU/kg. Gestational age was calculated from the estimated time of ovulation. A female rat was caged with a male between 17.00 and 09.00 h, and ovulation was estimated to have occurred at 01.00 h. Pregnant rats were detected by palpation 12.5 days later. Parturition occurred in our colony after 22 days of gestation. Pregnant females were TPTX by surgery under light ether anesthesia.

— *In the first set of experiments*, TPTX mothers were studied on days 17.5, 18.5, 19.5, 20.5 and 21.5 of gestation and compared to normals or to dietary controls of the same age. Since dietary intake decreased progressively from day 18 of gestation until term in TPTX mothers (see Results), we compared that group to normal females subjected to the same dietary restriction (dietary controls for TPTX animals).

— *In the second set of experiments*, four different groups of pregnant females were studied at day 21.5 of gestation : (i) sham-operated = normal females, (ii) dietary controls for TPTX animals, (iii) TPTX mothers, (iv) TPTX mothers injected with different doses of 1,25-$(OH)_2D_3$ (0.05-1 μg/kg of body weight) and compared with untreated TPTX mothers or sham-operated normal females. The treated animals received a daily subcutaneous injection of 1,25-$(OH)_2D_3$ (Hoffmann-La Roche, Basel, Switzerland) dissolved in absolute ethanol on days 17.5, 18.5, 19.5 and 20.5 of gestation.

The mothers were killed by decapitation between 09.00 and 10.00 h and a blood sample was collected. The fetuses were rapidly delivered and blood was withdrawn through an incision across the axillary vessels. The fetal liver was removed quickly and stored in frozen nitrogen. Since a rapid drop occurs in plasma calcium and phosphate levels soon after detachment of the fetus (Garel, 1969), a different protocol was used for the studies determining fetal plasma concentrations of calcium and inorganic phosphorus. Those fetuses, under light ether anesthesia, were excised one by one from the well-ventilated living mother by laparotomy and hysterectomy, leaving the placental circulation intact. Fetal blood samples were immediately obtained with heparinized Pasteur pipettes by section of the brachial vessels. At the end of the experiment, the maternal blood was removed by intracardiac puncture.

*Biochemical methods.* — Plasma calcium was estimated by flame photometry (Eppendorf) and plasma inorganic phosphorus was determined by the method of Chen, Toribara and Warner (1956). Blood barium hydroxyde-zinc sulfate filtrates were used for glucose determination with the glucose-oxidase method of Huggett and Nixon (1957). Liver glycogen was estimated by the method of Roehrig and Allred (1974) ; the frozen liver was weighed and homogenized at 0 °C in a Potter-Elvehjem tissue homogenizer. An aliquot was incubated 10 min at 55 °C with amylo-α 1,4-α 1,6 glucosidase (Boehringer, Mannheim, W. Germany) and then treated by glucose-oxidase.

*Statistical analysis.* — The data were analyzed using Student's unpaired t-test to determine the significance of changes between control and experimental groups. These data are presented as the means ± SEM.

## Results.

1. *Maternal thyroparathyroidectomy.*

*Effects on the mother.* — Before day 18 of gestation, dietary intake was the same in both normal and TPTX females, but decreased progressively in TPTX mothers from that day (11.4 ± 2.2 *vs* 19.0 ± 0.5 g in normals) until term (0.2 ± 0.1 g *vs* 16.0 ± 1.2 g in normals). In TPTX animals, food intake was 7.2 ± 2.3 at day 19 and 0.85 ± 0.3 g at day 20 of gestation as opposed to 17.1 ± 1.8 g and 16.4 ± 0.2 g, respectively, in normal females. The weight gain of both mothers and fetuses was about 45 g between days 12.5 and 19.5 of gestation in control and TPTX groups. No weight gain was observed during the last two days of gestation in the TPTX group, but the normal females gained 15 g.

Forty percent of the TPTX pregnant females died in tetany crisis at day 19.5 of gestation. Plasma calcium concentration in normal females slightly decreased between

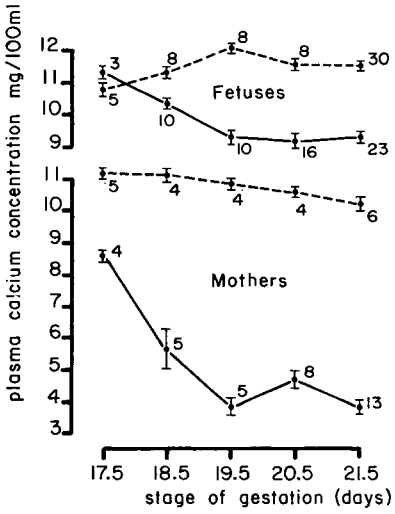


FIG. 1. — *Effects of maternal TPTX at day 12.5 of gestation on plasma calcium concentrations in mothers and fetuses.*

●---● : sham-operated normal females ; ●—● : TPTX mothers. Means ± SEM and the number of observations. $P < 0.001$ between TPTX and normals, except plasma calcium levels in 17.5-day-old fetuses ($P > 0.05$).

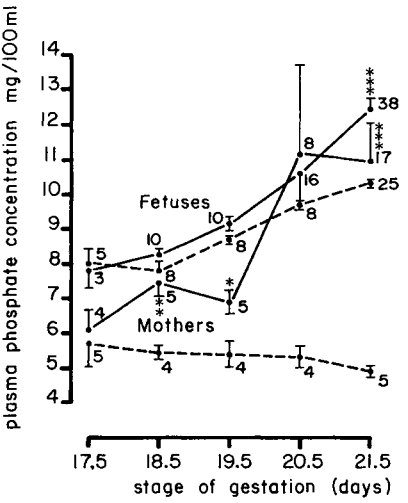


FIG. 2. — *Effect of maternal TPTX at day 12.5 of gestation on plasma phosphate levels in mothers and fetuses.*

●---● : sham-operated normal females ; ●—● : TPTX mothers. Means ± SEM and the number of observations. * : $P < 0.05$ from normals ; ** : $P < 0.01$ from normals ; *** : $P < 0.001$ from normals.

days 17.5 and 21.5 of gestation (— 0.9 mg/100 ml ; $P < 0.05$), as reported in an earlier study (Pic, 1969). A drop in plasma calcium was observed at day 21.5 of gestation in control females with dietary restriction (— 0.88 mg/100 ml ; $P < 0.001$). In TPTX mothers, plasma calcium was already significantly reduced on day 17.5 of gestation ; between days 19.5 and 21.5 of gestation, maternal plasma calcium remained constant at a low value : ~ 4 mg/100 ml (fig. 1). Plasma phosphate levels in normal mothers were unchanged during the last five days of gestation, as found in an earlier study by Garel and Pic (1972). In dietary control females, the plasma phosphate level was similar to that in the normal females. Plasma phosphate levels significantly increased 6 days after TPTX in the mothers (fig. 2) ; on day 21.5 of gestation, plasma phosphate in TPTX mothers was twice that in the normals.

*Effects on the fetus.* — The weight of fetuses from dietary control mothers was unchanged until day 19.5 of gestation (fig. 3), but decreased slightly at days 20.5 (— 0.22 g) and 21.5 (— 0.75 g) of gestation. The weight of fetuses from TPTX mothers, already significantly reduced on day 19.5 of gestation ($1.84 \pm 0.05$ vs $2.08 \pm 0.02$ g in dietary controls ; $P < 0.001$), decreased considerably on the following days (fig. 3). Dietary restriction in mothers had no effect on fetal plasma calcium and phosphate levels. The plasma calcium level in fetuses from TPTX mothers had already decreased significantly by day 18.5 of gestation ($P < 0.001$) (fig. 1). A marked shift in plasma calcium concentrations (— 2.7 mg/100 ml) was observed between fetuses from TPTX mothers and those from normal mothers on day 19.5 of gestation ; that difference was still evident on the last day of gestation (fig. 1). Plasma phosphate levels were higher in fetuses from TPTX mothers from days 18.5 to 21.5 of gestation (fig. 2) ; however, the difference was only significant on the last day.

2. *Effects of* 1,25-$(OH)_2D_3$ *injections into TPTX mothers.*

Injecting 0.025 μg of 1,25-$(OH)_2D_3$/kg of body weight into TPTX mothers had no effect (fig. 4), but administering doses between 0.05 and 1.00 μg/kg normalized the food intake and linearly increased (log-dose response) maternal plasma calcium. A normal plasma calcium level was reached in both mothers and fetuses at day 21.5 of gestation when 0.125 μg of 1,25-$(OH)_2D_3$/kg was used. Fetal weight, determined in parallel with these different doses at 21.5 days of gestation (fig. 4), greatly increased within a dose range of 0.05-0.25 μg/kg, when maternal plasma calcium was maintained between 8.5 and 12 mg/100 ml and fetal plasma calcium was normal. The use of higher doses ($\geqslant 0.5$ μg/kg) associated with severe maternal and fetal hypercalcemia ($\geqslant 13$ mg/100 ml) induced a marked inhibition of fetal weight. Subcutaneous calcifications were observed in the fetuses of some litters from TPTX mothers treated with 1 μg of 1,25-$(OH)_2D_3$/kg. Fetal blood glucose was normalized when TPTX mothers received 1,25-$(OH)_2D_3$ in doses ranging from 0.05 to 0.5 μg/kg. The increment in fetal blood glucose was partly inhibited after injection of 1 μg/kg (fig. 4). The liver glycogen stores in fetuses at day 21.5 of gestation were greatly increased with the use of 0.05 1,25-$(OH)_2D_3$/ kg (fig. 4) ; this rise was gradually inhibited by the use of higher doses. However, in allcases, fetal liver glycogen stores remained at higher levels than those in fetuses from untreated-TPTX mothers.

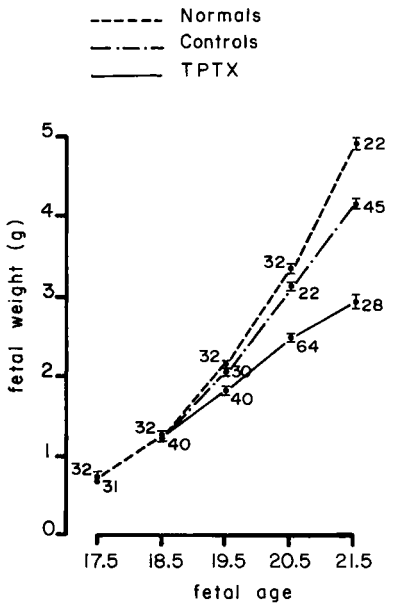


FIG. 3. — *Effect of maternal TPTX at day 12.5 of gestation on fetal weight.*

●---● : fetuses from sham-operated normal females ; ▲-·-·▲ : fetuses from dietary control mothers ; ●—● : fetuses from TPTX mothers. Means ± SEM and the number of animals. For statistical analysis between groups see « Results ».

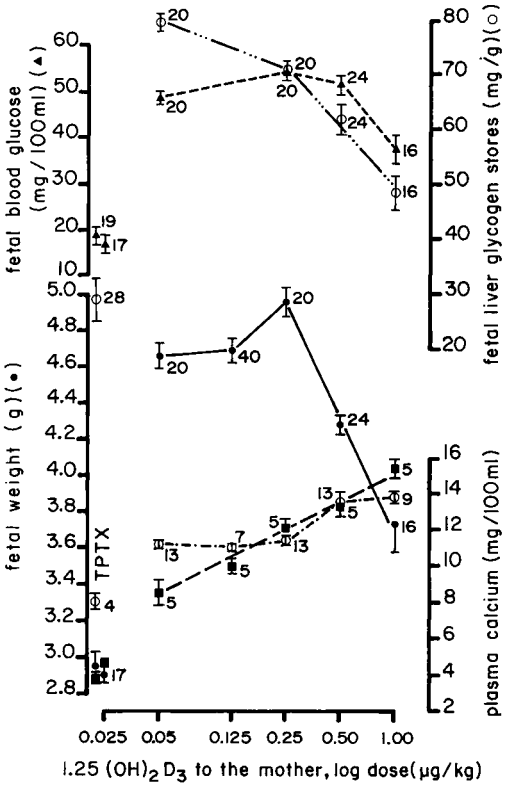


FIG. 4. — *Effect of* $1,25(OH)_2D_3$ *injections into TPTX mothers on maternal* (■----■) *and fetal* (o-.-o) *plasma calcium, fetal weight* (●—●), *fetal blood glucose* (▲---▲) *and liver glycogen stores* (o-...-o) *at day 21.5 of gestation.* Means ± SEM and the number of animals. TPTX : untreated TPTX mothers.

## Discussion.

From the present results, it appears that, after maternal TPTX, fetal weight had already decreased by day 19.5 of gestation, this reduction being considerably enhanced on the subsequent days. An increase in fetal weight (1.6-2.0 g) at 21.5 days of gestation was observed after the injection of $1,25\text{-}(OH)_2D_3$ into TPTX mothers at doses ranging from 0.05 to 0.25 µg/kg. This improvement was similar to that occurring after the graft of one parathyroid gland to TPTX mothers (Garel and Gilbert, 1978 ; Gilbert, Besnard and Garel, 1980). The use of larger doses reduced fetal weight, perhaps due to the toxic effects of $1,25\text{-}(OH)_2D_3$ or to chronic maternal and fetal hypercalcemia.

Decreases in maternal plasma calcium levels in TPTX mothers were paralleled by reduced fetal levels between days 17.5 and 19.5 of gestation, This decrease in the fetus might be related to the beginning of fetal ossification which occurs at day 17.5 of gestation (Comar, 1956 ; Jost, Moreau and Fournier, 1960 ; Chef, 1969) ; the amounts of calcium, transferred at the same time from the mother to the fetus, increased considerably (Comar, 1956 ; Chef, 1969). Thus a sharp decrease in maternal plasma calcium level induced chronic fetal hypocalcemia ($> 2$ mg/100 ml).

We showed that a low dose of 1,25-$(OH)_2D_3$ (0.05 μg/kg of body weight ; 30 picomoles per pregnant female) normalized fetal blood glucose and induced a 2.5-fold increase in fetal liver glycogen stores ; however, maternal plasma calcium remained low : 8.5 mg/100 ml. Whatever the maternal plasma calcium level, it appeared that both fetal blood glucose and liver glycogen levels were higher in 1,25-$(OH)_2D_3$-treated TPTX mothers than in untreated TPTX mothers.

These data suggest that the fetal weight in TPTX mothers was somewhat dependent on maternal blood 1,25-$(OH)_2D_3$ levels since we have shown that plasma 1,25-$(OH)_2D$ levels decrease in TPTX mothers on day 21.5 of gestation (Garel *et al.*, 1981). The requirements for 1,25-$(OH)_2D_3$ probably increase at the end of gestation ; the observation of elevated serum 1,25-$(OH)_2D_3$ levels in pregnant rats at the end of gestation (Halloran, Barthell and de Luca, 1979 ; Pike *et al.*, 1979 ; Garel *et al.*, 1981) may argue in favor of this hypothesis. It has been shown in lactating rats that plasma 1,25-$(OH)_2D_3$ levels are partly dependent on the presence of the parathyroid glands (Pike *et al.*, 1979). In our experiments, the effect of an elevated plasma calcium level on fetal growth cannot be dissociated from the effect of the active vitamin $D_3$ metabolite, but when maternal plasma calcium ranged from 8.5 to 12 mg/100 ml, fetal weight was markedly improved (around 4.6 g). The weight of newborn pups from vitamin D-deficient mothers was reported to be normal but litter size was reduced (Halloran and de Luca, 1979). This quite different model cannot exclude adaptation to the vitamin D-deficient state. In contrast, our experimental model represents the acute effects of disturbing mineral homeostasis at a critical period of gestation.

These experiments clearly demonstrate that low amounts of 1,25-$(OH)_2D_3$ are essential to fetal growth in hypoparathyroid pregnant rat mothers, but that higher doses have several toxic effects which are expressed in some litters by subcutaneous calcification. These deleterious effects are observed in the presence of hypercalcemia in both mothers and fetuses. Recently, Marx *et al.* (1980) reported that a woman with an hereditary decrease in 1,25-$(OH)_2D_3$ sensitivity, and treated during pregnancy with high doses of 1,25-$(OH)_2D_3$, gave birth to an apparently normal child. Since plasma calcium in women was not elevated during pregnancy, these observations in humans and rats suggest that high concentrations of 1,25-$(OH)_2D_3$ in maternal serum are not directly teratogenic, but that hypercalcemia *in utero* has teratogenic effects.

*Reçu en mars* 1981.
*Accepté en juillet* 1981.

*Acknowledgements.* — The 1,25-$(OH)_2D_3$ was kindly supplied by Hoffmann-La Roche (Basel, Switzerland). This work was supported by a grant from INSERM (ATP 56-78-88, contract N° 5).

**Résumé.** Le fœtus de mère thyroparathyroïdectomisée (TPTX) est caractérisé par un poids réduit, une calcémie et une glycémie basses et des réserves en glycogène hépatique effondrées, par contre sa phosphatémie est augmentée ; mais l'injection à la ratte TPTX de 1,25-$(OH)_2D_3$ permet de corriger ces effets. Dans le présent travail, on a analysé, sur ces différents paramètres fœtaux, les effets de l'injection de doses croissantes de 1,25-$(OH)_2D_3$ à la mère TPTX. Pour des doses de 1,25-$(OH)_2D_3$ comprises entre 0,05 et 0,25 μg/kg de poids corporel, le poids du fœtus à terme se trouve fortement augmenté (+ 1,6-2,0 g). Des doses supérieures (⩾ 0.5 μg/kg) associées à une forte hypercalcémie maternelle et fœtale inhibent progressivement cette augmentation. L'injection de 1 μg/kg de 1,25-$(OH)_2D_3$ entraîne l'apparition dans certaines portées de calcifications sous-cutanées chez le fœtus. La glycémie fœtale est normalisée par l'administration à la mère TPTX de doses de 1,25-$(OH)_2D_3$ comprises entre 0,05 et 0,5 μg/kg, pour une dose supérieure elle diminue. Une charge maximale du foie fœtal en glycogène s'observe pour une dose de 1,25-$(OH)_2D_3$ de 0.05 μg/kg, l'administration de doses plus élevées est associée à une inhibition progressive de cette charge en glycogène.

Ces résultats montrent clairement que, chez le rat, l'injection de doses physiologiques de 1,25-$(OH)_2D_3$ à la mère TPTX est accompagnée d'une reprise de la croissance fœtale, mais que par contre, l'administration de doses pharmacologiques a des effets toxiques sur le fœtus, ceci en présence à la fois chez la mère et le fœtus d'une hypercalcémie.

## References

CHEF R., 1969. Variation de la teneur en calcium et en phosphore du fœtus de rat en fonction de l'âge de la gestation. *C. R. Soc. Biol.*, **163,** 215-218.

CHEN P. S., TORIBARA T. Y., WARNER H., 1956. Microdetermination of phosphorus. *Analyt. Chem.*, **28,** 1756-1758.

COMAR C. L., 1956. Radiocalcium studies in pregnancy. *Ann. N. Y. Acad. Sci.*, **64,** 281-298.

GAREL J.-M., 1969. Action hypocalcémiante et hypophosphatémiante de la thyrocalcitonine chez le rat avant et après la naissance. *C. R. Acad. Sci. Paris*, **268,** *Sér. D*, 1525-1528.

GAREL J.-M., DELORME A., MARCHE P., NGUYEN T. M., GARABEDIAN M., 1981. Vitamin $D_3$ metabolite injections to thyroparathyroidectomized pregnant rats : effects on calcium binding proteins of maternal duodenum and of feto-placental unit. *Endocrinology*, **109,** 284-289.

GAREL J.-M., GELOSO-MEYER A., 1971. Hyperparathyroïdisme fœtal chez le rat consécutif à un hypoparathyroïdisme maternel. *Rev. europ. Etud. clin. biol.*, **16,** 174-178.

GAREL J.-M., GILBERT M., 1978. Rôle des parathyroïdes maternelles et des métabolites de la vitamine $D_3$ dans la croissance fœtale et la mise en réserve du glycogène dans le foie maternel et fœtal chez le rat. *C. R. Acad. Sci. Paris*, **286,** *Sér. D*, 1459-1461.

GAREL J.-M., GILBERT M., BESNARD P., 1979. Effects of vitamin $D_3$ metabolite injections into thyroparathyroidectomized pregnant rats on fetal weight and liver glycogen stores in mothers and fetuses, 271-274. In NORMAN A. W., SCHAEFER K., HERRATH D. V., GRIGOLEIT H. G., COBURN J. W., DE LUCA H. F., MAWER E. B., SUDA T., *Vitamin D basic research and its clinical application*. De Gruyter, Berlin.

GAREL J.-M., PIC P., 1972. Evolution of phosphatemia in the rat fetus during the late stages of gestation. *Biol. Neonate*, **21,** 369-374.

GILBERT M., BESNARD P., GAREL J.-M., 1980. Effects of maternal parathyroid glands and vitamin $D_3$ metabolites on fetal growth and fetal liver glycogen stores in thyro-parathyroidectomized pregnant rats. *Biomedicine*, **32,** 93-99.

HALLORAN B. P., DE LUCA H. F., 1979. Vitamin D deficiency and reproduction in rats. *Science*, **204,** 73-74.

HALLORAN B. P., BARTHELL E. N., DE LUCA H. F., 1979. Vitamin D metabolism during pregnancy and lactation in the rat. *Proc. nat. Acad. Sci. USA*, **76,** 5549-5553.

HUGGETT A. S. G., NIXON D. A., 1957. Use of glucose oxidase, peroxidase, and O-dianisidine in determination of blood and urinary glucose. *Lancet*, **2,** 368-370.

JOST A., MOREAU G., FOURNIER C., 1960. Date et ordre d'apparition des premiers centres d'ossification chez le fœtus de rat normal ou soumis au propylthiouracile. *Arch. anat. microsc. Morph. exp.*, **49**, 431-458.

MARX S. J., SWART E. G., HAMSTRA A. J., DE LUCA H. F., 1980. Normal intrauterine development of the fetus of a woman receiving extraordinarily high doses of 1,25-dihydroxyvitamin $D_3$. *J. clin. Endocrinol. Metab.*, **51**, 1138-1142.

PIC P., 1969. Evolution de la calcémie fœtale du rat en fin de gestation. *C. R. Soc. Biol.*, **159**, 1274-1277.

PIKE J. W., PARKER J. B., HAUSSLER M. R., BOASS A., TOVERUD S. U., 1979. Dynamic changes in circulating 1,25-dihydroxyvitamin D during reproduction in rats. *Science*, **204**, 1427-1429.

PORTERFIELD S. P., WHITTLE E., HENDRICH C. E., 1975. Hypoglycemia and glycogen deficits in fetuses of hypothyroid pregnant rats. *Proc. Soc. exp. Biol. Med.*, **149**, 748-753.

ROEHRIG K. L., ALLRED J. B., 1974. Direct enzymatic procedure for determination of liver glycogen. *Analyt. Biochem.*, **58**, 414-421.