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Vitamin A supply and biochemical development of the rat heart: studies on cellular DNA, RNA and protein

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Summary: The effect of restricted vitamin A supply to the mother on the biochemical development of the heart in rats has been studied. The vitamin A status of female rats was varied by supplying low, medium and adequate amounts (6, 40, 100 µg retinol/day/kg body weight respectively) of vitamin A during pregnancy and suckling. The vitamin A status of the mother caused an alteration in the developmental pattern of fetal heart in parameters of tissue weight, DNA, RNA and protein contents and the biosynthesis of DNA and protein from [^{3}H]-thymidine and [^{3}H]-leucine respectively. A dose-dependent effect of vitamin A on the metabolism of DNA, RNA and protein was observed in the developing rat hearts. Hence, emphasis should be given to the adequate availability of vitamin A to dams for the normal growth and development of fetal organs.

Zusammenfassung: Die Auswirkung einer beschränkten Vitamin-A-Zufuhr bei trächtigen Ratten auf die biochemische Entwicklung des fetalen Herzens wurde untersucht. Der Vitamin-A-Status der weiblichen Ratten wurde durch Angebot von geringen, mittleren und hohen Mengen an Vitamin A während Trächtigkeit und Säugeperiode verändert (6,40 und 100 µg Retinol/Tag/kg Körpergewicht). Der Vitamin-A-Status der Muttertiere verursachte eine Veränderung in der Entwicklung des fetalen Herzens in bezug auf Gewebegewicht, DNA-, RNA- und Proteingehalt sowie der Biosynthese von DNA und Proteinen aus jeweils [^{3}H]-Thymidin und [^{3}H]-Leucin. In den sich entwickelnden Rattenherzen wurde eine dosisabhängige Wirkung von Vitamin A auf den DNA-, RNA- sowie den Proteinstoffwechsel beobachtet. Eine angemessene Versorgung der Muttertiere mit Vitamin A ist entscheidend für die normale Entwicklung fetaler Organe.

Key words: vitamin A; development of fetal heart; DNA; RNA; protein; rat

Schlüsselwörter: Vitamin A; Entwicklung fetaler Organe, DNA; RNA; Protein; Ratte

Introduction

It is well documented that vitamin A is the essential micronutrient for reproduction, normal fetal growth, biochemical differentiation and development of mammalian cells (1–4). Studies have shown that severely vitamin A deficient female rats fail to conceive and mildly deficient

females conceive but usually abort or resorb the fetus in later gestation. In these circumstances malformed fetuses and pups are produced (1, 2).

No information is available as yet regarding the effect of maternal vitamin A deficiency on the biochemical development of the heart. However, Takahashi et al (2) have reported that the feeding of a vitamin A deficient diet to retinoic acid supplemented dams resulted in the failure of these rats to produce live and viable offspring. Normally the growth and biochemical development of organs in rats has been studied by employing DNA, RNA and protein parameters (5, 6). Since a total vitamin A deficiency model cannot be used to study the growth and development of fetus for obvious reasons, in the present study therefore, we have attempted to elucidate the effects of low, medium and adequate supply of vitamin A to the vitamin A deprived pregnant rats, on the DNA, RNA and protein contents and the biosynthesis of DNA from [^{3}H]-thymidine and protein from [^{3}H]-leucine respectively, in the developing hearts of rats.

Materials and Methods

Female Wistar strain rats (160–180 g) were kept on a vitamin A deficient diet (7) for 1 week. After 1 week of feeding the diet, the liver and plasma vitamin A levels were 27.5 ± 2.1 µg/g and 15.1 ± 1.6 µg/dl respectively. These females were mated with male

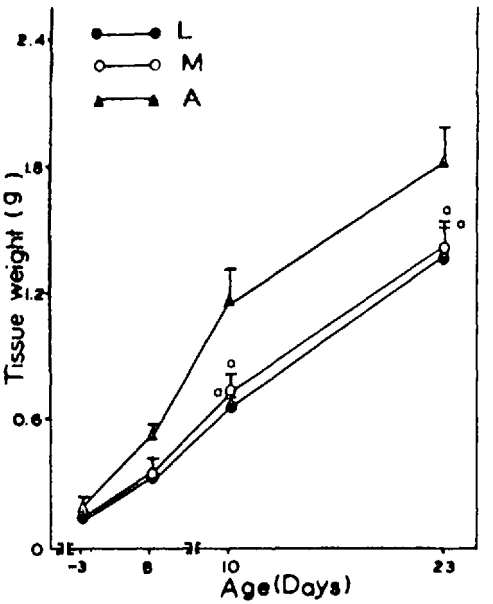

Fig. 1. Effect of maternal vitamin A restriction on the heart tissue weight of pups at various periods of development. Values are mean ± S.E.M. from three litters in each group. Vertical bars represent S.E.M. [a] and [b] show statistically significant values where $p \leq 0.05$. [a] = L vs A; [b] = L vs M. L = Low vitamin A (6 µg/day/kg body weight) supplemented group; M = Medium vitamin A (40 µg/day/kg body weight) supplemented group; A = Adequate vitamin A (100 µg/day/kg body weight) supplemented group.

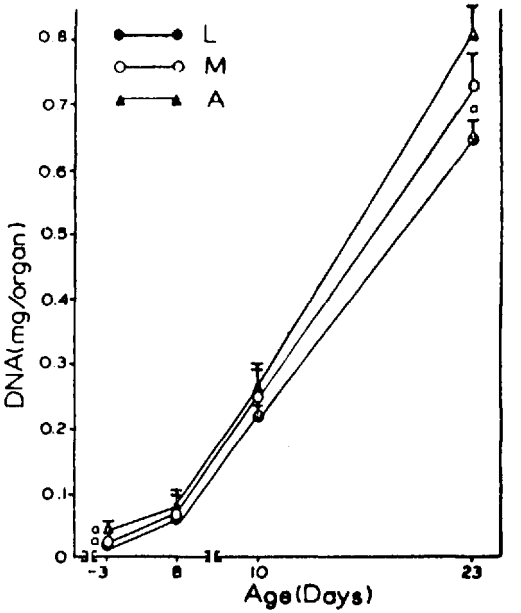

Fig. 2 DNA contents of the developing heart in relation to the maternal vitamin A status. Details as in Fig. 1.

Wistar strain rats. The period of conception was determined by the sperm positive test in the vaginal smears and this day was referred to as zero day of gestation. The pregnant rats were divided into three groups and pair-fed a vitamin A deficient diet with varying amounts of vitamin A supplementation: Group L: Low vitamin A (6 μg retinol/day/kg body weight); Group M: Medium vitamin A (40 μg retinol/day/kg body weight); Group A: Adequate vitamin A (100 μg retinol/day/kg body weight).

Three female rats with their litters from each group were sacrificed on the 20th day of gestation, or at birth, 10th and 23rd day of postnatal age (weaning). 3 h before sacrifice at the 20th day of gestation, each dam and at birth, day 10 and 23 of postnatal age each pup from each group were intraperitoneally injected (10 μCi/100 g body weight) with a saline solution of [^{3}H]-thymidine (specific act. 15.2 mCi/mmol) or [^{3}H]-leucine (specific act. 12 Ci/mmol) in separate experiments. Rats were sacrificed by decapitation and litters were collected surgically at the 20th day of gestation. The pups were sacrificed by cervical dislocation and their hearts were removed, cleaned, pooled (usually 3–4 in one sample) and processed for the isolation (8) and estimation of DNA, RNA and protein (9–11). Suitable aliquots of DNA and protein fractions were used for radioactivity determination in a Kontron MR-300 liquid scintillation counter.

Results and Discussion

Tissue weight

The heart weight increased fairly linearly with age of the pups. However, the tissue weight was significantly reduced at day 10 and 23 of postnatal age in pups from group L and M as compared to those from the group A

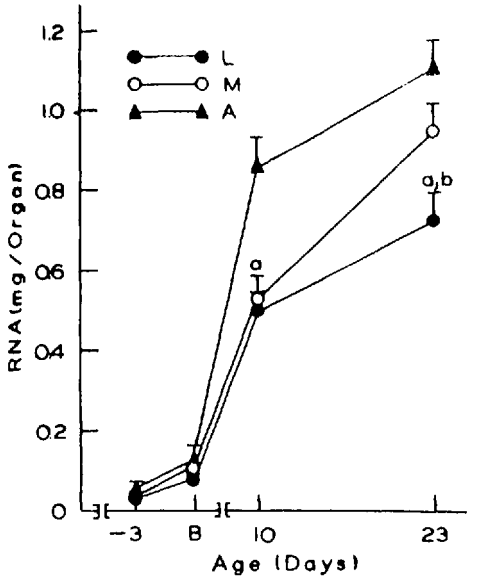

Fig. 3. Developmental pattern of RNA in the heart of pups in relation to the maternal vitamin A status. Details as in Fig. 1.

(Fig. 1). No significant difference in the heart weight was observed during gestation and suckling periods among the three groups as the demand of vitamin A for heart development is being met through the placenta and

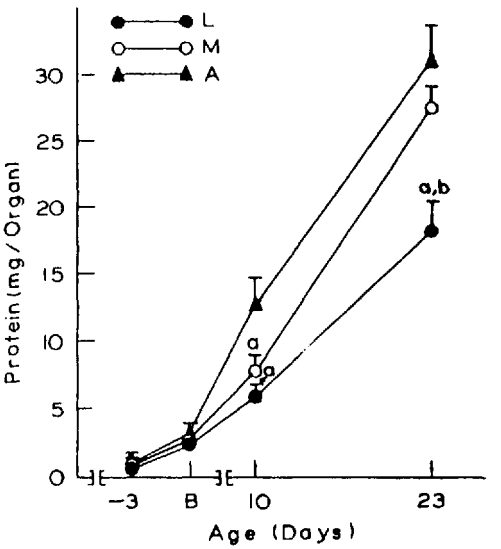

Fig. 4. Protein content of the developing heart in relation to the maternal vitamin A status. Details as in Fig. 1.

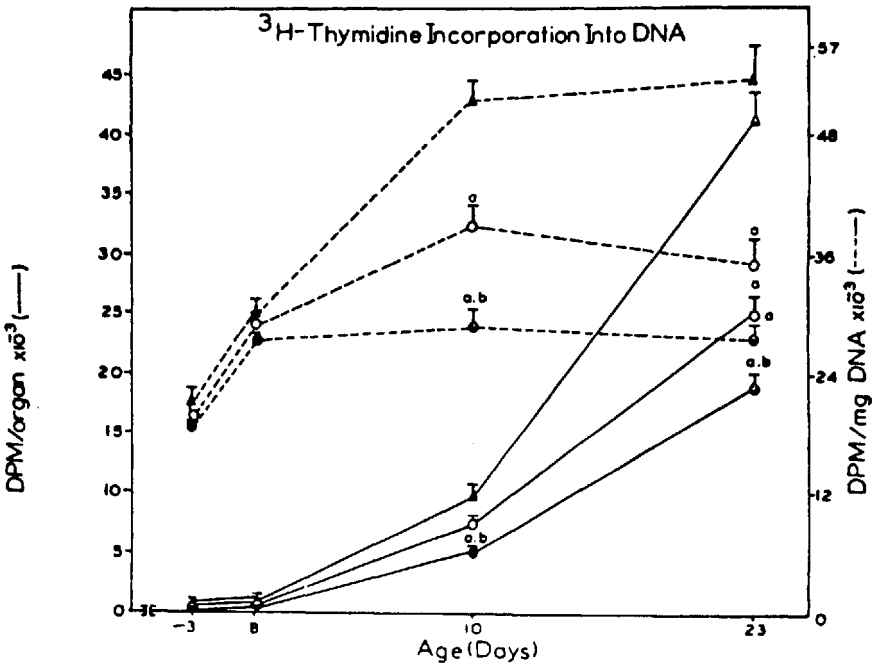

Fig. 5. Incorporation of [^{3}H]-thymidine into the heart DNA of pups at various periods of development in relation to maternal vitamin A status. Total activity is expressed as DPM/organ and the specific activity as DPM/mg DNA. (●——●) and (●···●) represent the L group; (○——○) and (○···○) represent the M group; (▲——▲) and (▲···▲) represent the A group. Other details are as described in Fig. 1.

maternal milk respectively. These results indicate an optimal requirement of vitamin A for the normal postnatal development of the heart.

DNA, RNA and protein contents

Developmental patterns of DNA, RNA and protein in the heart showed nearly linear increase in their contents with the age of the pups until the 23rd day of postnatal development in all three groups (Figs. 2–4). DNA content of the heart was significantly reduced on the 20th day of gestation and 23rd day of postnatal age of pups in group L as compared to groupA (Fig. 2). Maternal vitamin A restriction did not significantly affect the RNA and total protein contents of the heart during gestation; however, they were significantly reduced in the heart of pups from group L as compared to group A at day 10 and 23 of postnatal age (Figs. 3, 4). The reduced levels of DNA, RNA and protein in the heart of pups from group L are ascribed to the low maternal vitamin A intake.

Incorporation of labelled precursors into DNA and protein

The decreased amount of DNA, RNA and protein in the heart of pups of group L at various periods of development could be due either to the

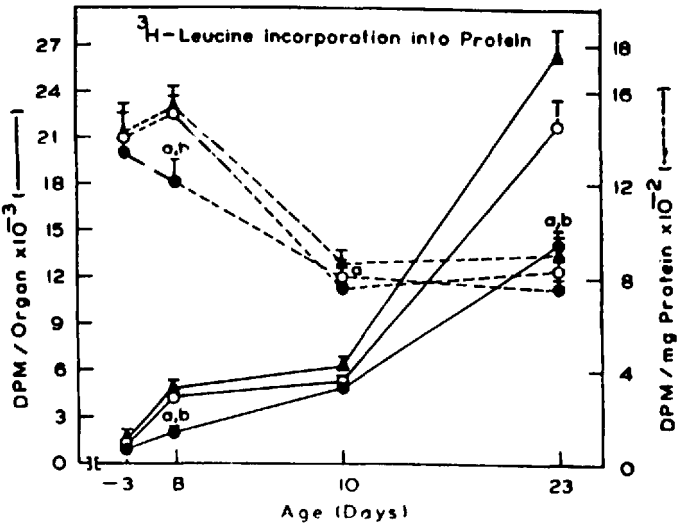

Fig. 6. Incorporation of [^{3}H]-leucine into the heart protein of pups at various periods of development in relation to maternal vitamin A status. Details are same as described in Fig. 1 and 5.

decreased anabolism, increased catabolism or both. To evaluate these possibilities, the incorporation of [^{3}H]-thymidine into DNA and [^{3}H]-leucine into protein of the developing fetal heart were studied and results are shown in Figs. 5 and 6. The pattern of synthesis (DPM/organ) of DNA and protein was nearly identical to that observed for their amounts. The turnover of DNA (DPM/mg DNA) was profoundly reduced in pups of group L as compared to groups M and A at all periods of postnatal growth. However, the turnover (DPM/mg protein) of proteins in the developing heart showed maximum value in groups M and A at birth with a decrease during suckling and showed a plateau after day 10 postnatally (Figs. 5, 6). The decrease in the specific activity (DPM/mg protein) in the heart of pups in group L may be attributed to the reduced cell proliferation and enhanced catabolism of protein, culminating in the reduced accumulation of protein in the heart.

These results thus show the vitamin A dependent metabolism of DNA, RNA and protein in the developing heart. The variations noted in DNA, RNA and protein levels of the heart during postnatal development are likely to be due to the differences in fetal hepatic vitamin A reserves (12). The reserves of vitamin A in the livers of pups derived from groups L and M were remarkably low as compared to those from group A, due to the low maternal vitamin A status and/or low availability of vitamin A through milk and further non-availability of it in the diet.

Our results strongly suggest that the low availability of vitamin A during the development of the fetus markedly affects the differentiation, development and maturation process in the rat heart and hence its expression of biochemical functions in the later life, which may be reversible or irreversible.

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