Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 26:116–124 (1987)

Biochemical development of the rat lung: Studies on cellular DNA, RNA and protein content in relation to maternal vitamin A status

H. S. Sharma and U. K. Misra

Department of Biochemistry, V. P. Chest Institute, Delhi University, Delhi, India

Summary: The effect of maternal vitamin A restriction on the biochemical development of the lung in rats has been studied. The vitamin A status of dams has been varied by supplying low, medium and adequate amounts of vitamin A (6, 40 and 100 μg retinol/day/kg body weight, respectively) during pregnancy and suckling. The results demonstrate that the restricted supply of vitamin A to the mother affected the growth and development of the lung in parameters of tissue weight, DNA and protein levels and their biosynthesis from respective labelled precursors, RNA contents, cell number and cell size.

Zusammenfassung: Der Einfluß einer Beschränkung der Vitamin-A-Zufuhr für das Muttertier auf die biochemische Entwicklung der Lunge des Jungtieres wurde bei Ratten untersucht. Der Vitamin-A-Status der Muttertiere wurde durch niedrige, mittlere und adäquate Gaben von Vitamin A während Trächtigkeit und Säugezeit variiert (6, 40 und 100 μg Retinol/Tag/kg Körpergewicht). Die Ergebnisse zeigen, daß die Beschränkung der Vitamin-A-Zufuhr für das Muttertier das Wachstum und die Entwicklung der Lunge in bezug auf Gewicht, DNA- und Proteingehalt und -biosynthese aus markierten Vorstufen, RNA-Gehalt, Zellgröße und Zellzahl beeinflussen.

Key words: lung, development, vitamin A, DNA, RNA, protein, rat

Introduction

At birth, after interruption of the fetal placental circulation, the newborn infant has to assume vital functions previously sustained by the mother. Consequently, because of the urgent need to achieve effective gas exchange, the lung is a crucial organ in early adaptation to the extrauterine life. The lung in the embryo of rat appears as a laryngotracheal groove in the form of an outgrowth from the foregut in early gestation (5–7 days) (1). Pre- as well as postnatal development of the rat lung has been studied by a number of workers (2–5). Gain in weight of the whole body or in any tissue has been commonly used as a measure of growth and development. Nevertheless, this approach might be misleading, since body weight may rise even in the absence of growth. Therefore, DNA, RNA and protein levels of the cell have been employed by many

workers as biochemical parameters of normal growth and development of any particular organ (6–8). Cell number and cell size calculated from the tissue weight and its DNA content have also been used as parameters of growth for developing organs.

Vitamin A deficiency among the children of an underprivileged population is quite prevalent in many areas of the world. Several studies have provided evidence that vitamin A influences the biochemical development and differentiation of mammalian cells (9–11). In a study on rats, Takahashi et al. (10) reported that the feeding of a vitamin A deficient diet to the retinoic acid supplemented dams resulted in reduced levels of DNA, RNA and protein in the developing fetuses. Earlier we have reported that maternal vitamin A restriction impairs the accumulation of DNA, RNA and protein in the whole fetus and liver in rats (12, 13). In the present investigation, we studied the effects of low, medium and adequate vitamin A supplementation to the mother, on DNA, RNA and protein content of the developing fetal lung in rats.

Materials and Methods

Chemicals

Deoxyribonucleic acid, ribonucleic acid and bovine serum albumin were procurred from M/S Sigma Chemicals Co., St. Louis, Missouri, United States. All other chemicals and reagents used in this study were of analytical reagent grade.

Radioactive chemicals

^{3}H-leucine (specific activity 12 Ci/mmol) and ^{3}H-thymidine (specific activity 15.2 mCi/mmol) were obtained from Bhabha Atomic Research Centre, Bombay, India.

Experimental animals

Wistar strain female rats (160–180 g) from the Institute-maintained colony were kept on a vitamin A deficient diet (14) for a period of 1 week. The rats were housed in individual cages with free access to water. The liver and plasma vitamin A levels after 1 week of feeding were assessed as 27.5 ± 2.1 μg/g and 15.1 ± 1.6 μg/dl respectively. On eighth day of feeding, these females were mated with normal male rats of the same strain. The period of conception was determined by the sperm positive test in the vaginal smears and this day was taken as day 0 of gestation. The pregnant rats were divided into the following three groups on the basis of vitamin A supplementation and were pair-fed a vitamin A deficient diet. Group L: Low vitamin A supplementation (6 μg retinol/day/kg body weight); Group M: Medium vitamin A supplementation (40 μg retinol/day/kg body weight); Group A: Adequate vitamin A supplementation (100 μg retinol/day/kg body weight).

The treatment was continued until weaning. Three dams from each group, with their litters, were sacrificed on the 20th day of gestation or at birth, 10th or 23rd postnatal day (weaning). Three hours before sacrifice each dam and pup from the each group were intraperitoneally injected with a sterile saline solution of ^{3}H-thymidine or ^{3}H-leucine (10 μCi/100 g body weight) in separate experiments. The pups were injected through a very fine needle attached to microsyringe. Dams were sacrificed by decapitation and litters were collected surgically at the 20th day of gestation. The pups were sacrificed by cervical dislocation and their lungs were quickly removed, cleaned, pooled (3–4 tissues in one sample) and processed for the isolation of DNA, RNA and protein.

Isolation of DNA, RNA and protein

A 10 % (w/v) homogenate of the tissue in ice-cold distilled water was made, and DNA and RNA were extracted according to the method of Munro and Fleck (15). Proteins were isolated by 10 % trichloroacetic acid and were delipidized with ethyl alcohol : diethyl ether (2:1, v/v) mixture. DNA was estimated by diphenylamine reaction (16), RNA by the orcinol reaction method (17) and proteins were measured according to the method of Lowry et al. (18). Suitable aliquots of DNA and protein fractions were taken in scintillation vials for radioactivity determination in Kontron MR-300 liquid scintillation counter using a dioxane based scintillation cocktail (19).

Results and Discussion

Tissue weight

The lung weight increased linearly with age up to the 10th postnatal day and thereafter reached a stationary value in all three groups (Fig. 1). The developmental pattern shows that the low intake of vitamin A by dams significantly reduced the lung weight in the progeny, as compared to those receiving an adequate amount of vitamin A during gestation and suckling. These results demonstrate the necessity for an optimal amount of vitamin A for the growth and development of the lung.

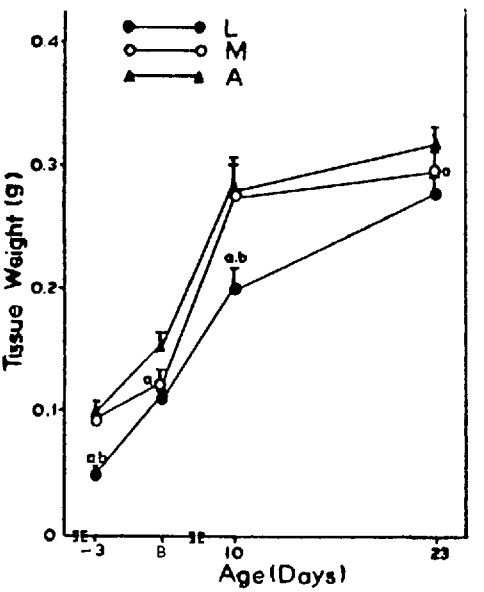

Fig. 1. Effect of maternal vitamin A restriction on lung tissue weight of pups at various periods of development. Values are mean ± S.E.M. from three litters in each group. Vertical bars represent the S.E.M. [a] and [b] show the statistically significant values where $p \leq 0.05$. [a] = L vs A; [b] = 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.

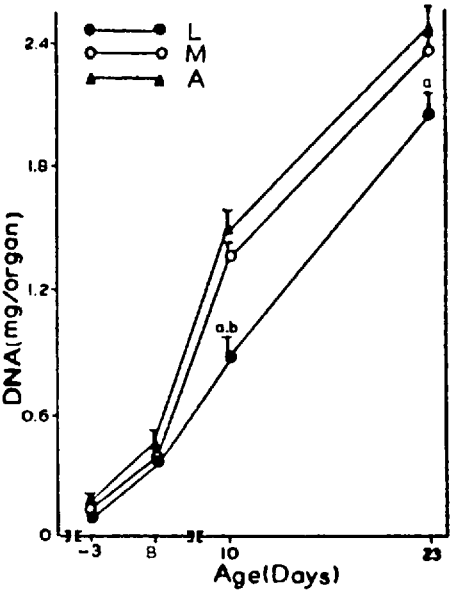

Fig. 2. DNA content of the developing lung in relation to maternal vitamin A status. Details as in Fig. 1.

DNA, RNA and protein content

The DNA and protein contents (mg/organ) of the lung were found to increase linearly with age of pups in all three groups (Figs. 2, 4). The developmental pattern of RNA was somewhat different because it increased fairly linearly until the 10th day of age and then it "plateaued

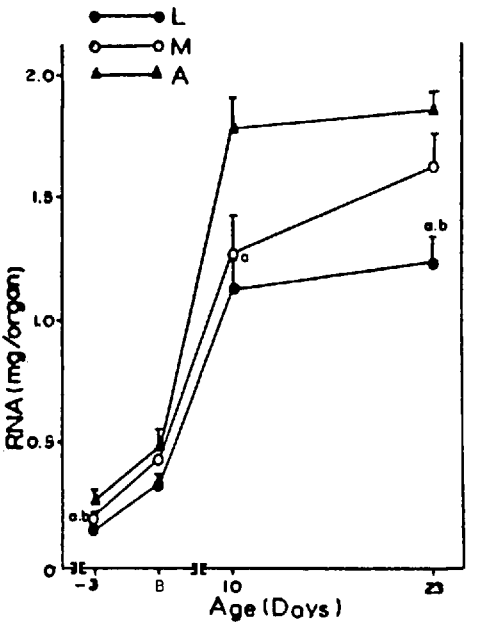

Fig. 3. RNA content of the developing lung in relation to maternal vitamin A status. Details as in Fig. 1.

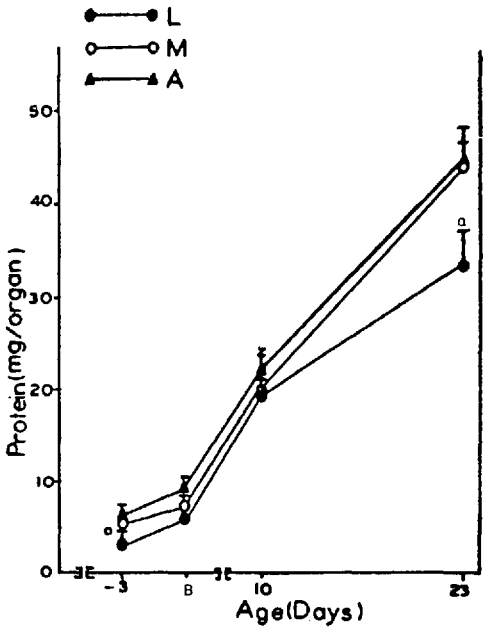

Fig. 4. Protein content of the developing lung in relation to maternal vitamin A status. Details as in Fig. 1.

off" in groups L and A, whereas in group M, the RNA content was further increased after 10 days after birth (Fig. 3). It was observed that the maternal vitamin A restriction profoundly affected the DNA, RNA and protein contents of the developing lung. The DNA content was not significantly influenced by the maternal vitamin A restriction during gestation; however, if was significantly decreased in group L as compared to group A at day 10 and 23 of postnatal age (Fig. 2). The lung RNA and protein contents were significantly reduced in group L as compared to group A at the 20th day of gestation and weaning, but at the 10th postnatal day only the RNA content was significantly decreased in group L as compared to group A (Figs. 3, 4). These results show the vitamin A dependent metabolism of DNA, RNA and proteins in the developing lung. The significant differences observed in DNA, RNA and protein levels in the lung during its postnatal development could possibly be ascribed to the differences in fetal hepatic vitamin A stores (20, 21). The total vitamin A hepatic reserves of pups derived from groups L and M were remarkably low as compared to the pups derived from group A, as a consequence of a very low intake of vitamin A through milk and, furthermore, the nonavailability of vitamin A from the nibbling diet, which was vitamin A free.

Cell number and cell size

To elucidate the effect of maternal vitamin A restriction on the developmental pattern of pulmonary cells, the cell number (number of nuclei) and

Table 1. Developmental pattern of rat pulmonary cells in relation to maternal vitamin A status.

Period	Parameter	Dietary groups		
		L	M	A
20th day of gestation	Cell number*	11.13 ± 0.84[a,b] (18)	11.45 ± 1.32 (23)	15.65 ± 0.81 (28)
	Cell size**	4.85 ± 0.38[a,b]	7.42 ± 0.53	7.39 ± 0.62
Birth	Cell number	57.09 ± 9.67 (18)	61.62 ± 6.77 (20)	70.05 ± 9.84 (20)
	Cell size	2.84 ± 0.17	2.59 ± 0.08	2.47 ± 0.19
10th day postnatal	Cell number	140.16 ± 17.17[a,b] (11)	220.65 ± 29.35 (15)	235.16 ± 16.13 (18)
	Cell size	1.43 ± 0.12	1.45 ± 0.09	1.44 ± 0.08
23rd day postnatal	Cell number	332.09 ± 11.12[a,b] (6)	379.91 ± 21.74 (10)	394.35 ± 12.90 (9)
	Cell size	1.25 ± 0.08	1.20 ± 0.08	1.23 ± 0.09

Values are mean ± SEM from the number of pups shown in parentheses.
* Cell number is expressed as number of nuclei × 10^{-6}.
** Cell size is expressed as weight/nucleus (ng).
[a] and [b] show statistically significant values ($p \leq 0.05$).
[a] = L vs A; [b] = L vs M.

cell size (weight/nucleus) were calculated and the results are shown in Table 1. The cell number increased and cell size decreased with the growth of lung (Table 1). Similar observations have also been reported by other workers (8, 22). It is evident from these results that the maternal vitamin A restriction significantly reduced the cell number in the lung of its progeny at the 20th day of gestation, 10th and 23rd postnatal day. Cell size was comparable in three groups at all the periods of growth, except at the 20th day of gestation, where it was significantly reduced in the pups from group L, as compared to those from groups M and A (Table 1).

Incorporation of labelled precursors into DNA and protein

To evaluate the influence of maternal vitamin A status on the synthesis and breakdown of DNA and protein in lung, the incorporation of ^{3}H-thymidine into DNA and ^{3}H-leucine into protein was studied and the results are shown in Figs. 5 and 6. The developmental pattern of incorporation of these precursors (dpm/organ) into DNA and protein were nearly identical to that observed for their amounts. The results demonstrate that the low maternal intake of vitamin A significantly reduced DNA and protein synthesis (dpm/organ) in the lung during its pre- and postnatal development. The developmental pattern of the specific activity (dpm/mg protein) which represents the turnover of DNA in lung was similar to that of the developmental pattern of DNA content; however, it was significantly reduced at the 20th day of gestation and the 10th postnatal day in

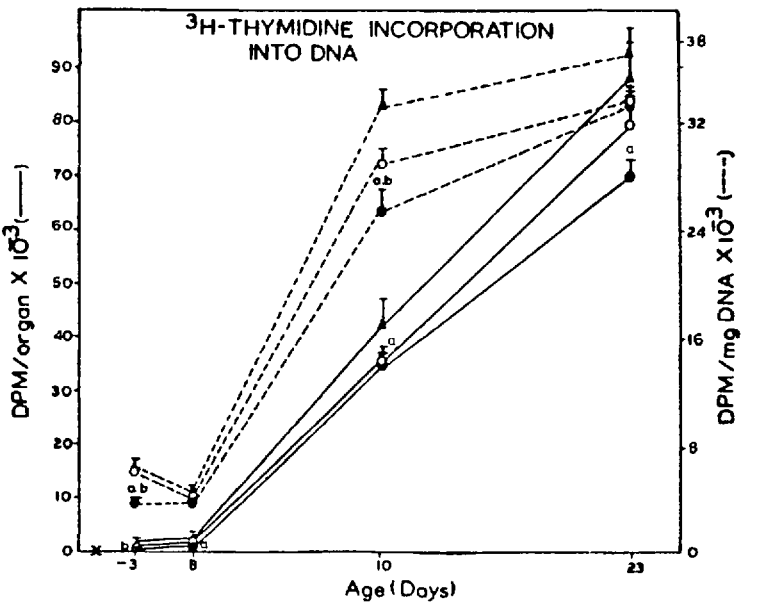

Fig. 5. Incorporation of ^{3}H-thymidine into lung DNA of pups at various periods of development in relation to maternal vitamin A status.
Total activity is expressed as dpm/organ and the specific activity as dpm/mg DNA.
(●——●) and (●----●) represent the L group;
(○——○) and (○----○) represent the M group;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

the pups obtained from group L, as compared to those from groups M and A (Fig. 5). Unlike DNA, the turnover of lung proteins sharply decreased until the 10th day of age in all three groups, and thereafter it remained almost constant (Fig. 6). This indicates the enhanced rate of catabolism during gestation and suckling, resulting in the reduced accumulation of protein in the developing lung.

Our results on the biochemical development of the lung are in agreement with those of other workers (4, 8); however, the lung maturation was profoundly affected by the low intake of vitamin A by the mother. The reduced levels of DNA, RNA and protein in the lung during development of pups in group L could be attributed to reduced cellular differentiation, as reflected by the reduced cell number (Table 1). These results indicate that the growth of the fetal lung and the metabolism of DNA, RNA and protein appear to be dependent on the availability of vitamin A to the dams during pregnancy and suckling. The literature available on maternal vitamin A status and fetal malformations arising from it, and the results of the present investigation, provide further support for the action of vitamin A in cellular differentiation and growth. The results also show that the effects of maternal vitamin A deficiency appear to be reversible and most of the aberrated biochemical functions may be recovered after supplemen-

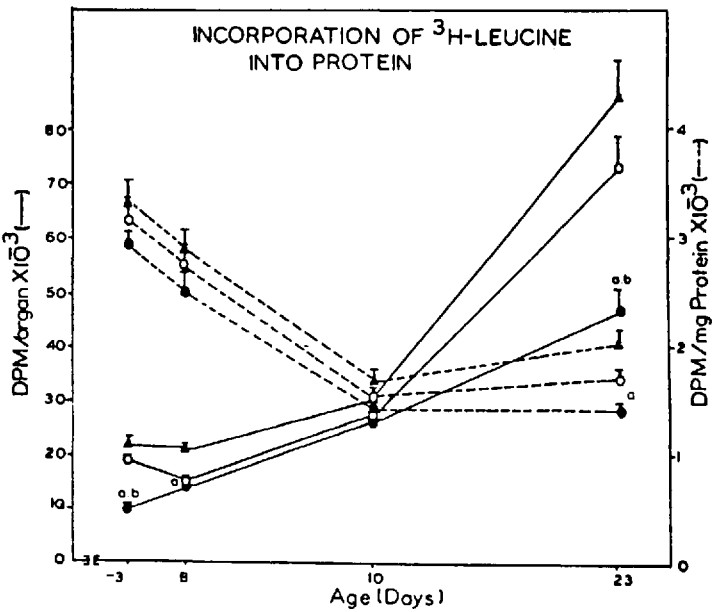

Fig. 6. Incorporation of ^{3}H-leucine into lung protein of pups at various periods of development in relation to maternal vitamin A status. Details are as described in Figs. 1 and 5.

tation of adequate vitamin A before the onset of irreversible damage. The mechanism of action of vitamin A in pre- and postnatal development, though, is not yet clear. Its adequate supply in the diet of the pregnant mother appears to be a "must" for the normal growth and development of the lung.

Acknowledgements

Financial assistance from the Indian Council of Medical Research is gratefully acknowledged. Vitamin A was generously provided by Hoffmann-La Roche, Bombay (India).

References

1. Perelman SM, Engle MJ, Farrell PM (1981) Perspective of fetal lung development. Lung 159:53–80
2. Passmayer F (1982) The perinatal lung. In: Jones CT (ed) Biochemical development of the fetus and neonate. Elsevier Biomedical Press, pp 337–391
3. Winick M, Noble A (1965) Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. Develop Biol 12:451–466
4. Kistler A (1979) Tissue specific changes in DNA, RNA and protein contents during late fetal and postnatal development in the rat. Int J Biochem 10:975–980
5. Hamosh M, Simpson MR, Mullen DK, Hamosh P (1981) Metabolic activity of developing rat lung. Biol Neonate 39:117–126

6. Mirsky AE, Ris H (1949) Variable and constant components of chromosomes. Nature 163:666–667
7. Enesco M, Leblond CP (1962) Increase in cell number as a factor in growth of organs of the young male rat. J Embryol Exp Morphol 10:530–562
8. Winick M, Brasel JA, Rosso P (1972) Nutrition and cell growth. In: Winick M (ed) Nutrition and development. Willey Interscience, New York, pp 49–99
9. Wilson JG, Roth CS, Warkany J (1953) An analysis of syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A during gestation. Am J Anat 92:189–217
10. Takahashi YI, Smith JE, Winick M, Goodman DS (1975) Vitamin A deficiency and fetal growth and development in the rat. J Nutr 105:1299–1310
11. Shukla RR, Joshi HC, Misra UK (1983) Developmental pattern of DNA and proteins in brain, liver, lung and heart of rats given excess vitamin A postnatally. Biol Neonate 44:243–250
12. Sharma HS, Misra UK (1985) Developmental pattern of the hepatic DNA, RNA and protein in relation to maternal vitamin A status in rats. Acta Vitam Enzymol 7:85–92
13. Sharma HS, Misra UK (1987) Effect of maternal vitamin A restriction on the fetal DNA during prenatal development of the rat. Nutr Rep Int 35:561–566
14. Malathi P, Subarao K, Sastry PS, Ganguly J (1963) Studies on metabolism of vitamin A. I: The biological activity of vitamin A acid in rats. Biochem J 87:305–311
15. Munro HN, Fleck A (1966) The determination of nucleic acids. Methods Biochem Anal 15:113–176
16. Burton K (1956) A study of the conditions and mechanisms of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315–323
17. Dische Z (1955) The nucleic acids. In: Chargaff E, Davidson JN (eds) Vol 1. Academic press, New York
18. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
19. Hattori T, Aoki H, Mitsuzaki I, Maruo B, Takahashi H (1965) Liquid scintillation counting of ^{3}H-nucleic acids. Anal Chem 37:159–161
20. Sharma HS, Misra UK (1986) Postnatal distribution of vitamin A in liver, lung, heart and brain of the rat in relation to maternal vitamin A status. Biol Neonate 50:345–350
21. Sharma HS, Sriram K, Misra UK (1986) Vitamin A accumulation in the fetal tissues during prenatal development of the rat in relation to maternal vitamin A status. Nutr Rep Int 34:221–228
22. Kauffman SL (1980) Cell proliferation in the mammalian lung. Int Rev Exp Pathol 22:132–191

Received April 15, 1987

Authors' address:

Hari S. Sharma, Max-Planck-Institut für biophysikalische Chemie, Abteilung Molekulare Biologie, 3400 Göttingen (West Germany)