Effect of Vitamin A Nutritional Status on the Ribonucleic Acids of Liver, Intestinal Mucosa and Testes of Rats

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1. Vitamin A deficiency led to an increase in the oligonucleotide fraction of testes and intestinal mucosa of rats at the expense of high-molecular-weight RNA and 4S RNA, but no such changes were observed in the liver. Retinyl acetate supplementation reversed these effects in both tissues, whereas retinoic acid supplementation was almost equally effective in the mucosa but virtually ineffective in the testes. The ribonuclease activities of all the tissues remained unaffected by the above treatments. 2. The effect of vitamin A deprivation on the acceptor activity of the tRNA of the testes and intestinal mucosa was more pronounced than on the liver tRNA. The testes and mucosal tRNA of the retinoic acid-supplemented rats showed significantly lower charging capacity as compared with the retinyl acetate-supplemented ones. Here also no significant effect was observed on the liver tRNA. 3. Vitamin A deficiency caused a decrease in the percentage of poly(A) in RNA of the mucosa and testes, but not in the liver RNA. The poly(A) contents of both tissues were brought to normal by retinyl acetate supplementation; treatment with retinoic acid led to an appreciable increase in poly(A) in the mucosa, but considerably less increase in poly(A) in the testes. 4. The incorporation of $H_3^{32}PO_4$ into the rRNA and tRNA of the testes was lowered by vitamin A deficiency, but no such effects were observed in the liver RNA.

Although the mechanism through which vitamin A functions in the visual system is fairly well established, its systemic mode of action is still little understood. But critical examination of the extensive information accumulated in the literature leads to the general conclusion that it is directly involved in the general process of growth of higher animals. Thus the most reliable and accurate method of assay of very small amounts of vitamin A is still based on the growth response of vitamin A-deficient rats. Indeed, probably deprivation of no other essential nutrient exerts such pronounced effects on the growth of the rat as that caused by deprivation of vitamin A, and at the same time supplementation with no other nutrient causes such prompt restoration of growth of the deficient animals as that brought about by vitamin A. Ganguly (1974) has reviewed many such examples and has suggested that deprivation of vitamin A affects first those cells that rapidly proliferate in rats under normal conditions, and Jayaram *et al.* (1975) have produced some evidence in support of such an idea. Regenerating rat liver, after partial hepatectomy, has been widely recognized as a model for growth and rapid cell proliferation. By using such a system Jayaram *et al.* (1975) have demonstrated that regeneration of rat liver is markedly less in the vitamin A-deprived animals, but it can be restored to normal by supplementation of the deficient rats with retinyl acetate immediately after the partial hepatectomy.

We have previously shown that the first and rate-limiting enzyme in steroidogenesis, i.e. the cholesterol side-chain-cleavage enzyme, is markedly affected in the testes and ovaries, but not in the adrenals, of vitamin A-deprived rats, and have pointed out that this may be due to the differences in the rates of regeneration of the cells in these tissues (Jayaram *et al.*, 1973). In the rat the rates of differentiation and division vary in other cells also. Thus there are several rapid steps of mitosis during spermatogenesis; the mucosal cells regenerate every 24–36 h whereas the rate of regeneration of the liver cells is much lower. In the present work we have attempted to compare the effects of depletion of vitamin A on the metabolism of RNA species of these three tissues, and we show that, even at a very mild stage of vitamin A deficiency, significant changes occur in the RNA of those cells that rapidly proliferate.

Materials and Methods

^{32}P -labelled H_3PO_4 and ^{14}C -labelled algal protein hydrolysates (specific radioactivity 13.1 mCi/mg-atom of carbon) were obtained from the Bhabha Atomic Research Centre, Bombay, India.

Phenol supplied by British Drug Houses, Bombay, India, was freshly distilled, after which water was added to make it 80% (w/v).

Ribonuclease-free deoxyribonuclease was obtained from Worthington Biochemical Corp., Free-

hold, N.J., U.S.A.; [^3H]poly(U) (specific radioactivity 3.2×10^5 c.p.m./ μg) was from Miles Research Laboratories, Kankakee, IL, U.S.A.; non-radioactive poly(A) was supplied by Sigma Chemical Co., St. Louis, MO, U.S.A.

Treatment of animals

Male albino rats of this Institute strain were maintained after weaning on the vitamin A-deficient diet of Malathi *et al.* (1963) until they just stopped gaining weight. At this stage some of them were killed, but others continued on the same vitamin A-deficient diet and received daily supplements of retinyl acetate or retinoic acid at the rate of $40 \mu\text{g}/\text{rat}$ per day for the given periods of time. At the same time another batch of weanling male rats was kept on the deficient diet and simultaneously received supplements of retinyl acetate at the rate of $40 \mu\text{g}/\text{rat}$ per day throughout the experiment; these are called 'normal' rats. Throughout this work the vitamin A-deprived rats were used at a stage of depletion at which their food intake was not perceptibly affected. Therefore use of pair-fed controls was considered to be not necessary and the rats fed *ad lib.* served as controls.

All rats were killed by decapitation, after which the tissues were immediately transferred to cold 0.9% NaCl. Thereafter all operations were carried out at 0–4°C. After the intestinal contents had been washed out with several lots of cold 0.9% NaCl the mucosa was scraped off with the blunt end of a scalpel.

Extraction of RNA

Total RNA was extracted from the tissues by the phenol method of Kirby (1956), with the buffer modification described by Anandaraj & Cherayil (1974) (it contained 0.5% bentonite). Any DNA present in the RNA samples was removed by deoxy-ribonuclease treatment. The final RNA preparation was washed twice with ethanol, followed by several washings with diethyl ether, after which it was dried and dissolved in 0.05M-Tris/HCl buffer, pH 7.2, containing 0.14M-NaCl. As judged by the A_{260}/A_{280} ratio (>1.6) the RNA thus obtained was free from proteins. An A_{260} unit of 2 was taken as equivalent to $100 \mu\text{g}$ of RNA/ml.

The RNA used for the poly(A) assays was prepared by the same procedure, except that 0.05M-Tris/HCl buffer, pH 7.6, containing 0.14M-NaCl was used for the extraction of the RNA.

Isolation of tRNA

Here the total RNA isolated from the tissues was stirred for 15–20 min in 1M-NaCl (1.5–2 ml of solution/10 mg of RNA), after which the suspension was centrifuged at 5000g for 15 min, and the super-

natant was kept. This process was repeated once more and the RNA was precipitated from the combined supernatants by the addition of 2 vol. of ethanol at -20°C . The tRNA preparation was deacylated (von Ehrenstein, 1967), reprecipitated with ethanol and finally dissolved in 0.05M-Tris/HCl, pH 7.2, containing 0.14M-NaCl. The tRNA thus isolated from the testes was used directly for the amino acid-acceptor assay, but those isolated from the liver and intestinal mucosa were further purified by DEAE-cellulose chromatography (Anandaraj & Cherayil, 1974).

tRNA acceptor assay

The procedure described by Prasada Rao & Cherayil (1974) was followed for these assays, except that the incubations were carried out in test tubes (and not on filter-paper discs) for 10 min, after which suitable portions were transferred to filter-paper discs. In these experiments the crude homologous aminoacyl-tRNA synthetases isolated from the tissues of normal animals (Taylor *et al.*, 1968) were used, e.g. the liver enzyme was used for the assay of the liver tRNA acceptor activity.

Assay of ribonuclease activity

This activity was assayed in the postmitochondrial as well as in the postmicrosomal supernatant fractions of tissue homogenates. The reaction mixtures contained, in a total volume of 0.35 ml of 0.05M-Tris/HCl buffer, pH 7.0, ^{32}P -labelled RNA (specific radioactivity 42000 c.p.m./ μg of RNA) and approx. $700 \mu\text{g}$ of enzyme protein. After incubation for 45 min at 37°C , 0.1 ml portions of the reaction mixture were spotted on Whatman no. 3 filter-paper discs and dried. Subsequent treatments were as described under 'tRNA acceptor assay'. Similar samples from the control tubes (to which no enzyme was added) were also spotted on Whatman no. 3 filter-paper discs and processed as described above. The enzyme activity was expressed on the basis of the difference between the radioactivity retained on the control filter-paper discs and those containing the samples.

Determination of the poly(A) contents

The poly(A) contents of the total RNA isolated from the tissues were determined by the procedure of Gillespie *et al.* (1972), which consisted of hybridization of radioactive poly(U) with non-radioactive RNA, followed by digestion with ribonuclease. The ribonuclease-resistant poly(U) was precipitated, trapped on Millipore membranes and counted for radioactivity.

Counting of radioactivity

All radioactivity measurements were made in a Beckman LS-100 liquid-scintillation counter by using 0.5% 2,5-diphenyloxazole in toluene as the scintillation fluid.

The efficiencies of counting of the ^{14}C - and ^{32}P -labelled samples on Whatman no. 3 filter-paper discs were 75–80% and 90% respectively; on Millipore membranes the ^3H -labelled samples were counted with 25% efficiency, and the efficiency for ^{32}P -labelled RNA in polyacrylamide gels was approx. 60–65%.

Determination of proteins

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Results

Table 1 summarizes the changes in the total RNA contents of the liver, testes and intestinal mucosa of the rats with different vitamin A nutritional status. Although the total RNA contents were slightly lower in the vitamin A-deficient livers, when expressed per g of tissue they were comparable with those of the normal liver, because the liver weights were also lower in the deficient animals. In contrast, the total weight and total RNA contents of the testes were markedly decreased in the deficient animals. But, here also, when expressed per g of tissue there was very little difference between the two groups. The intestinal mucosa also showed a slight decrease in the total weight and total RNA contents, which were not as marked as in the testes, and the values expressed per g were comparable in the two groups.

After supplementation of the depleted rats with retinyl acetate or retinoic acid there was an increase in the wet weight and total RNA contents of the liver and mucosa of both groups, which reflected the growth of the animals during the period of supplementation for 30 days. However, although there was very little difference in the weights or total RNA of the two tissues between the two groups, the total weights and total RNA contents of the testes of the retinoate-supplemented rats were about 50% of the retinyl acetate-supplemented ones. Here also, in all cases the RNA values expressed per g of tissue were more or less comparable within a particular tissue.

The results of fractionation of the total RNA of the three tissues (Table 2) show that, although the contents of all the three fractions were comparable for liver RNA of all the groups, there was a definite increase in the oligonucleotide fractions ($P < 0.05$) of both mucosa and testes of the deficient animals, with a concomitant decrease in the high-molecular-weight and 4S RNA (presumably tRNA) fractions. Supplementation with retinyl acetate for 30 days restored

Table 1. *Effect of vitamin A nutritional status on the RNA contents of the liver, testes and intestinal mucosa of rats*

After being on the different dietary regimens the rats were killed, after which the RNA of the tissues was extracted and analysed as described in the text. The results are expressed as means $\pm$ s.d. of the values from five different animals. The tissue weights and RNA contents are per whole liver and per pair of testes, and for the mucosa the values correspond to the total mucosa obtained from each animal.

Treatment	Liver			Testes			Mucosa		
	RNA			RNA			RNA		
	Wet wt. (g)	(mg)	(mg/g of tissue)	Wet wt. (g)	(mg)	(mg/g of tissue)	Wet wt. (g)	(mg)	(mg/g of tissue)
Normal diet	5.76 $\pm$ 0.62	26.6 $\pm$ 3.1	4.63 $\pm$ 0.36	1.27 $\pm$ 0.22	4.00 $\pm$ 0.67	3.16 $\pm$ 0.10	3.36 $\pm$ 0.17	13.8 $\pm$ 0.83	4.12 $\pm$ 0.34
Vitamin A-deficient diet until depletion	4.38 $\pm$ 0.31	19.5 $\pm$ 2.7	4.65 $\pm$ 0.27	0.54 $\pm$ 0.05	1.82 $\pm$ 0.12	3.40 $\pm$ 0.22	2.97 $\pm$ 0.13	12.7 $\pm$ 0.35	4.13 $\pm$ 0.59
Supplemented with retinyl acetate for 30 days after depletion	8.26 $\pm$ 0.18	36.4 $\pm$ 3.9	4.40 $\pm$ 0.35	1.32 $\pm$ 0.10	4.42 $\pm$ 0.44	3.34 $\pm$ 0.22	4.34 $\pm$ 0.20	15.4 $\pm$ 0.91	3.59 $\pm$ 0.23
Supplemented with retinoic acid for 30 days after depletion	8.21 $\pm$ 0.14	38.6 $\pm$ 2.7	4.72 $\pm$ 0.37	0.71 $\pm$ 0.07	2.15 $\pm$ 0.16	3.01 $\pm$ 0.46	4.12 $\pm$ 0.50	15.2 $\pm$ 0.53	3.67 $\pm$ 0.20

Table 2. Fractionation of the RNA isolated from the tissues of rats

Total RNA (40 A_{260} units) isolated from the tissues was loaded on Sephadex G-100 columns (65 cm $\times$ 1.5 cm), after which the columns were eluted with Tris/HCl buffer (0.05 M, pH 7.5) containing 0.14 M-NaCl, and 3.2 ml fractions were collected. The RNA samples were resolved into three peaks corresponding to high-molecular-weight RNA, 4S RNA and degraded RNA materials (eluted at 1 column volume of the eluent and designated here as the oligonucleotide fraction). For measurement of the A_{260} units of the three peaks, the corresponding fractions were pooled. The results are expressed as A_{260} units per 100 A_{260} units of total RNA, and the values are means $\pm$ S.D. for four separate experiments.

Treatment	Liver			Mucosa			Testes		
	High-molecular-weight RNA	4S RNA	Oligo-nucleotides	High-molecular-weight RNA	4S RNA	Oligo-nucleotides	High-molecular-weight RNA	4S RNA	Oligo-nucleotides
Normal	65.3 $\pm$ 5.2	15.2 $\pm$ 2.4	9.71 $\pm$ 2.0	59.8 $\pm$ 5.0	16.2 $\pm$ 3.1	8.92 $\pm$ 2.0	61.4 $\pm$ 6.1	14.9 $\pm$ 2.9	10.6 $\pm$ 2.3
Deficient diet until plateau stage	63.6 $\pm$ 6.0	15.4 $\pm$ 3.0	10.3 $\pm$ 2.1	50.2 $\pm$ 4.6	12.5 $\pm$ 2.3	17.9 $\pm$ 4.6*	51.2 $\pm$ 5.8	11.2 $\pm$ 3.0	18.9 $\pm$ 3.7*
Supplemented with retinyl acetate for 30 days after mild deficiency	64.9 $\pm$ 4.9	14.4 $\pm$ 2.1	10.4 $\pm$ 1.6	58.6 $\pm$ 4.2	15.7 $\pm$ 3.4	9.84 $\pm$ 3.0	59.7 $\pm$ 5.3	14.4 $\pm$ 2.6	12.5 $\pm$ 2.4
Supplemented with retinoic acid for 30 days after mild deficiency	67.3 $\pm$ 6.2	14.0 $\pm$ 2.6	9.95 $\pm$ 2.2	56.7 $\pm$ 5.2	14.9 $\pm$ 3.2	12.1 $\pm$ 3.4*	51.5 $\pm$ 6.2	12.1 $\pm$ 2.4	18.2 $\pm$ 2.9*

* $P < 0.05$. Significance was according to Student's t test. The P values were between the normal and deficient rats and between retinyl acetate- and retinoic acid-supplemented rats.

the three fractions to normal values in both tissues; retinoic acid supplementation was almost as effective in the mucosa, but virtually ineffective in the testes.

Ribonuclease activity of the tissues

Assay of the ribonuclease activity of the three tissues showed that it was not affected by any of the treatments (results not shown).

^{32}P incorporation into the RNA of liver and testes

After injection of $H_3^{32}PO_4$ the specific radioactivities of the RNA isolated from the post-mitochondrial supernatant of the liver and testes of the deficient rats were approx. 90 and 70% respectively of those of the normal controls (results not shown). Electrophoretic analysis on 2.6% and 10% polyacrylamide gels of the ^{32}P -labelled RNA thus isolated showed that, although the deficiency did not have any significant effect on the incorporation of ^{32}P into the rRNA and tRNA of the liver (results not shown), the radioactivity of the rRNA and tRNA in the deficient testes showed a marked decrease (Fig. 1).

Amino acid-acceptor activities of the tRNA species

Table 3 shows that, in contrast with the liver tRNA, the acceptor ability of the tRNA from the intestinal mucosa and testes of the deficient rats was significantly lower ($P < 0.05$) than in the normal tissues. Moreover, the tRNA from the mucosa and testes of the retinoic acid-supplemented rats showed lower charging ability ($P < 0.05$) than that of the retinyl acetate-supplemented animals.

Changes in the poly(A) contents of the RNA

Table 4 shows that, although the percentage of poly(A) in the liver RNA was comparable in all the groups, vitamin A deficiency led to a definite decrease in the poly(A) contents of the RNA of the testes and mucosa ($P < 0.05$). Although retinyl acetate supplementation restored to normal the contents of poly(A) in RNA of both tissues, retinoic acid supplementation had less effect in the mucosa and very little effect in the testes.

Discussion

It has been indicated in the introduction that the extensive information available in the literature permits a generalization that deprivation of vitamin A first affects the rapidly proliferating cells of the animals, which implies that vitamin A might be necessary for rapid cell proliferation. Although the basic knowledge of the events that take place at the molecular level during the process of cell division is

Fig. 1. *Electrophoretic patterns of the ^{32}P -labelled postmitochondrial RNA of testes on polyacrylamide gel*

Each rat was given, intraperitoneally, 1 mCi of ^{32}P and was killed 3 h later. The liver and testes of three animals were pooled within each group, after which the RNA was extracted from the postmitochondrial supernatant fraction of the tissues and subjected to gel electrophoresis. (a) The preparation of 2.6% gels and electrophoresis were as described by Mirault & Scherrer (1971). After the run the gels were carefully placed on a Whatman no. 1 filter paper and the paper was folded over the gel, pressed and dried. The dried papers were cut into 2 mm strips along the length of the gels and counted for radioactivity. ^{32}P -labelled rat liver 28S and 18S rRNA was used as a marker. The RNA load was 6.3 A_{260} units/gel. ○, Normal; ●, vitamin A-deficient. (b) The 10% gels were prepared as described by Loening (1967) and the electrophoresis was carried out at 6 mA per tube with Bromophenol Blue as the tracking dye. After the run the gels were cut into 2 mm slices, dried on filter-paper discs and the radioactivity was counted. The marker used was ^{32}P -labelled rat liver 4S RNA and the load was the same as in (a). ○, Normal; ●, vitamin A-deficient.

very inadequate, it appears reasonable to assume that the flow of information in such processes must follow the sequence DNA $\rightarrow$ RNA $\rightarrow$ protein. Since RNA has been assigned a pivotal position in such a sequence of events, the present experiments were

designed to compare the effects of deprivation of vitamin A on the metabolism of RNA of some cells that regenerate rapidly with those of others that are much slower in regeneration in the same animal under normal conditions, and for this purpose the mucosa

Table 3. *Acceptor activity of the tRNA isolated from the tissues of differently treated rats*

The activity was assayed as described in the text. The values are means $\pm$ s.d. of duplicate assays for the tRNA from five animals in each group and are expressed as c.p.m./ A_{260} unit of tRNA.

Treatment	Liver	Testes	Mucosa
Normal diet	6780 $\pm$ 465	2020 $\pm$ 120	6250 $\pm$ 320
Deficient diet until weight plateau stage	6520 $\pm$ 518	1542 $\pm$ 141*	5860 $\pm$ 125*
Supplemented with retinyl acetate for 30 days after mild deficiency	9436 $\pm$ 625	2240 $\pm$ 148	6200 $\pm$ 210
Supplemented with retinoic acid for 30 days after mild deficiency	9178 $\pm$ 492	1980 $\pm$ 130*	5750 $\pm$ 315*

* $P < 0.05$; for explanation see footnote to Table 2.

Table 4. *Effect of vitamin A nutritional status on the percentage of poly(A) in the RNA of liver, testes and intestinal mucosa of rats*

A standard hybridization mixture contained, in 200 ml of 50% (v/v) formamide/3 $\times$ SSC (0.45 M-NaCl/0.045 M-sodium citrate): 0.01 M-Tris/HCl (pH 7.2); 0.06 μ g of poly(U) (sp. radioactivity 3.2×10^5 c.p.m./ μ g); 50–80 μ g of non-radioactive total RNA isolated from the tissues. After incubation for 3 h at 36°C the reaction mixtures were cooled to 30°C and 2 ml of a solution containing 0.01 M-Tris/HCl (pH 7.2), 0.01 M-MgCl₂, 0.05 M-NaCl, 5 μ g of pancreatic ribonuclease/ml and 20 μ g of electrophoretically pure deoxyribonuclease/ml was added to all samples, except for the ribonuclease-negative blank, to which a similar solution but lacking ribonuclease was added. All tubes were kept at 32°C for 2 h, after which 100 μ l of a solution containing 100 μ g of *Escherichia coli* 16S RNA was added followed by 1 ml of 32% (w/v) trichloroacetic acid. The tubes were kept in ice for 10 min. The trichloroacetic acid-insoluble precipitate was collected on Millipore filters and washed with cold 5% trichloroacetic acid followed by ethanol and ether, after which they were dried and counted for the radioactivity. The ribonuclease-negative blank showed the absence of spurious hydrolysis of the radioactive poly(U), and therefore no correction was necessary. The values are expressed as percentage of poly(A) with respect to the total RNA and are means $\pm$ s.d. of four independent assays.

Treatment	Liver	Testes	Intestinal mucosa
Normal	0.040 $\pm$ 0.004	0.050 $\pm$ 0.006	0.052 $\pm$ 0.003
Deficient diet until plateau stage	0.036 $\pm$ 0.003	0.032 $\pm$ 0.003*	0.038 $\pm$ 0.002*
Supplemented with retinyl acetate for 30 days after mild deficiency	0.034 $\pm$ 0.005	0.052 $\pm$ 0.005	0.054 $\pm$ 0.006
Supplemented with retinoic acid for 30 days after mild deficiency	0.034 $\pm$ 0.003	0.034 $\pm$ 0.002*	0.045 $\pm$ 0.003

* $P < 0.05$; for explanation see footnote to Table 2.

and testes of rats were chosen as examples of the former type, and the liver represented the latter.

In these experiments the rats were used at a stage at which they had just exhausted their initial supply of vitamin A and did not show any of the pathological effects that usually develop as a result of the secondary effects of prolonged deprivation of vitamin A. Even if there were some effects, these should cancel out, because the primary aim of this work was to compare the effects of vitamin A deficiency on the different types of cells of the same animal. Moreover, such considerations should not be applicable in the retinoic acid-supplemented rats, which have been universally accepted to be free from such effects of the deficiency.

By using such animals it is shown here that depletion of vitamin A in young male rats leads to a significant decrease in the high-molecular-weight RNA and 4S RNA of the mucosa and testes, but not of the liver. In all these tissues the total RNA, when expressed per g of tissue, was virtually the same in the normal as in depleted animals. However, on fractionation of the RNA isolated from these tissues on Sephadex G-100 columns the high-molecular-weight

and 4S RNA fractions from the deficient mucosa and testes were found to be less, with a concomitant increase in the oligonucleotide fractions, but no such changes were observed in the liver RNA. The experiments in which the ³²P-labelled RNA was analysed on polyacrylamide gels have also shown markedly decreased incorporation of the radioactivity into the rRNA and tRNA as well as into the other RNA fractions of the deficient testes, as compared with the deficient liver. De Luca *et al.* (1971) have shown that the decreased incorporation of radioactive uridine into the rRNA and tRNA of the intestinal mucosa of vitamin A-deficient rats was actually due to a decrease in the rate of synthesis and not to any differences in the nucleotide pool sizes. However, in our experiments the possibility that the lowered incorporation of ³²P into the RNA of the testes of the deficient animals might be due to lower uptake of the radioactivity by the tissue cannot be ruled out.

The increase in the oligonucleotide fraction at the expense of the high-molecular-weight and 4S RNA of the deficient mucosa and testes might conceivably be due to increased ribonuclease activities in the deficient tissues. But we have found no significant

changes in the ribonuclease activity of the post-mitochondrial or postmicrosomal supernatant of the normal and deficient tissues.

However, the amino acid-acceptor activities of the tRNA of the testes and intestinal mucosa were consistently lower in the deficient animals. Similarly the tRNA of the testes and mucosa of the retinoic acid-supplemented rats showed lower charging capacity compared with the retinyl acetate-supplemented animals. It is possible that the marked effects of vitamin A deficiency on the tRNA of these tissues may be due to some impairment of the tRNA methylase activity (Krause *et al.*, 1975).

The present observation that vitamin A deficiency affects the percentage of poly(A) in the RNA of the testes and mucosa but not of the liver of rats is also interesting. Poly(A) tracts have been shown to be present at the 3'-end of the heterogeneous nuclear RNA (hnRNA), which is the actual precursor for the mRNA, as well as of the cytoplasmic mRNA molecules. Poly(A) has also been implicated in the processing of hnRNA, in the stability of mRNA molecules and in the efficiency of their translation. It is therefore possible that vitamin A might act at the level of processing of the precursor RNA molecules to functional mRNA. In fact Kaufman *et al.* (1972) have produced evidence which strongly suggests that deprivation of vitamin A interferes with the synthesis of the rRNA precursors or their processing to the 28S and 18S RNA in the trachea of hamsters.

These results are in general agreement with the observations by earlier workers on the effect of vitamin A deficiency on RNA metabolism in rats. Thus Johnson *et al.* (1969) have reported stimulation of incorporation of radioactive uridine into the nuclear RNA of the liver and intestinal mucosa of vitamin A-deficient rats given retinoic acid, and Zile & De Luca (1970) claimed that vitamin A enhances RNA synthesis in several tissues of vitamin A-deficient rats, the effect being more marked in the colon and intestinal mucosa than in the liver. On the other hand, according to De Luca *et al.* (1971), vitamin A deficiency leads to a decrease in the synthesis of rRNA and tRNA of the intestinal mucosa of rats, without having any effect on the precursor nucleotide pool. The observations by Kaufman *et al.* (1972) are more pertinent here because, by electrophoretic analysis of the RNA of the tracheal epithelia of the vitamin A-deficient and pair-fed hamsters, they have demonstrated that deprivation of vitamin A causes a decrease in the RNA of low electrophoretic mobility, which might represent the precursor rRNA molecules. They have also shown that such changed patterns could be corrected by supplementation with retinyl acetate, which agrees with our present results. Therefore, on the whole, the earlier reports as well as ours show that the RNA of those cells that proliferate more rapidly is affected

most by the vitamin A nutritional status of the animals.

It should be emphasized here that the planning of these experiments was such that all rats of comparable age and weight could not be used, because those rats that were supplemented with retinyl acetate or retinoic acid for 30 days after the deficiency were naturally older and bigger than the deficient ones. Probably such considerations are reflected in the increased acceptor activities of the tRNA species of the liver of the retinyl acetate- and retinoic acid-supplemented rats, as compared with the normal rats.

One may also point out here that it has been widely accepted that retinoic acid can replace retinol in all respects of physiology of higher animals, except that the visual systems do not function and that female rats resorb their foetuses, and the testes do not develop in the males. But we have earlier shown that the ovaries of the retinoate-supplemented rats synthesize and secrete smaller amounts of the steroid hormones such as pregnenolone and progesterone (Ganguly *et al.*, 1971), and we have further shown (Jayaram *et al.*, 1975) that in the regenerating rat liver after partial hepatectomy retinoic acid is markedly less effective than retinol in reversing the effects of vitamin A deficiency. In the present work also it has been shown that retinoic acid is ineffective in reversing the effects of the deficiency on the RNA of rat testes, but is almost as effective as retinyl acetate in correcting those on the RNA of the intestinal mucosa. Therefore our present results lend further support to the earlier claims that retinol is more specifically required in the differentiation of the germinal epithelia, but the other types of epithelia may respond equally effectively to retinoic acid (Sporn *et al.*, 1976; Bonanni *et al.*, 1973).

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