Effect of Vitamin A Deficiency on the Protein Synthetic Activity of Rat Liver Ribosomes (35402)

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The protein synthetic apparatus of living cells is affected by vitamin A deficiency. DeLuca and co-workers (1, 2) have reported that lack of vitamin A directly impairs protein synthesis by bound ribosomes in the intestinal mucosa of the rat and that the synthesis of a specific glycoprotein is missing. These observations prompted us to investigate the effect of vitamin A deficiency on protein synthesis by rat liver ribosomes. This communication reports on the *in vitro* capacity of ribosomes from vitamin A-deficient animals to make protein in the presence and absence of a synthetic messenger RNA (mRNA).

Materials and Methods. Wistar strain male rats were made vitamin A deficient as previously described (3). Polyribosomes were prepared from rat liver essentially as described by Blobel and Potter (4). The pelleted material sedimenting through the 2.0 *M* sucrose was stored at —60° and used in protein synthetic experiments within 24 to 36 hr.

The 17,000*g* postmitochondrial supernatant (4) was further centrifuged at 144,880*g* for 2 hr and the supernatant was diluted with 2 vol of a medium containing 0.90 *M* sucrose, 0.004 *M* Mg acetate, 0.07 *M* KCl and 0.006 *M* 2-mercaptoethanol. The pH was then adjusted to 5.1 with 0.1 *M* acetic acid; and the precipitate obtained by centrifugation was once washed with cold, double-distilled water. The pellet was stored at —20° and used within 24 to 36 hr.

Protein synthesis assays were performed as described previously (5–7). Incubation was carried at 37° and, in this case, 1.0 mg of ribosomes, 2.4 mg of enzyme protein (pH 5.1); and 0.4 μCi of L-[^{3}H]-phenylalanine (sp act 1.65 Ci/mmole; Schwartz BioResearch, Inc.) were included in 1.1 ml of incorporation system. Incorporation of radioactivity into hot TCA-insoluble protein was determined by the Mans and Novelli method (8), as described (6, 7).

Protein was estimated by the Lowry method (9). Ribosome concentration was estimated using an OD_{260} of 1.0 equal to 90 μg of ribosomes, as reported (10).

Results and Discussion. Figure 1 shows the results of protein synthetic experiments using subcellular fractions from normal and vitamin A-deficient animals in the presence and absence of a synthetic mRNA (poly U). In all cases, the biological activity of the vitamin A-deficient system is higher than that of the normal system.

Figure 2 shows the results of hybrid experiments in which subcellular fractions, *i.e.*, ribosomes and pH 5.1 enzymes from normal and deficient animals, were crossed. The increased protein synthetic capacity of the vitamin A-deficient system is due to the pH 5.1 fraction. The amount of radioactivity incorporated by ribosomes from normal animals was higher with the pH 5.1 fraction from vitamin A-deficient animals than with the pH 5.1 fraction from normal animals. On the other hand, the protein synthetic activity of ribosomes from deficient animals was always higher in the presence of homologous pH 5.1 enzyme than in the presence of pH 5.1 enzymes from normal animals. Similar results were obtained when hybrid experiments were run with synthetic mRNA (polyuridylic acid). These observations suggest that vitamin A deficiency affects either the aminoacyl-tRNA synthetase enzymes and/or liver tRNA or an as yet unknown factor(s) which precipitates at pH 5.1 and stimulates *in vitro* synthesis.

The data presented here are in agreement

with the finding of De Luca *et al.* (1) that there is an increase in the protein synthetic activity of liver ribosomes and pH 5.1 enzymes from Vitamin A-deficient animals when compared with ribosomes and pH 5.1 enzymes from normal animals. We have demonstrated that the increased activity is due to the pH 5.1 enzyme fraction.

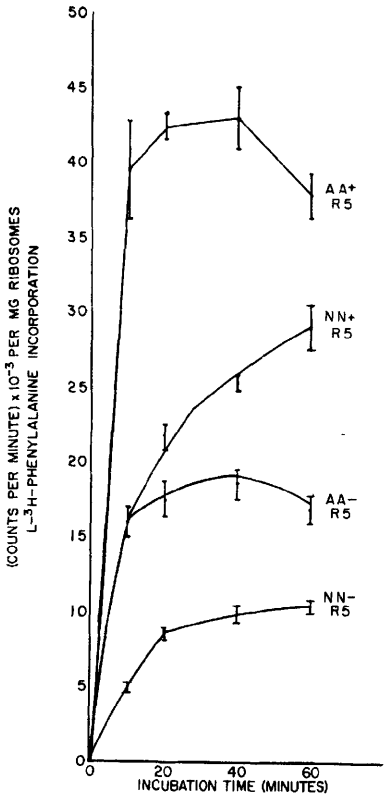

FIG. 1. Time-course of L-^{3}H-phenylalanine incorporation *in vitro* by liver ribosomes from normal and vitamin A-deficient animals in the presence and absence of polyuridylic acid. Protein synthesis assays were performed as described previously (5–7). Incubation was carried out at 37°. All poly U assays were run with preincubated ribosomes (40 min, 37°) as described (7, 11, 12). Aliquots of 100 μl were removed at the indicated time intervals, placed on 2.3-cm filter paper discs, dried and extracted as described by Mans and Novelli (8). Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. The vertical bars represent ± standard error of the mean. Each point is the average of 6 duplicate experiments (12 determinations). The concentration of polyuridylic acid (Sigma) was 100 μg (6, 7). N_RN_5 and A_RA_5 = ribosomes and pH 5.1 fractions from normal and vitamin A-deficient animals, respectively. (+) and (—) signs denote the presence and absence of polyuridylic acid.

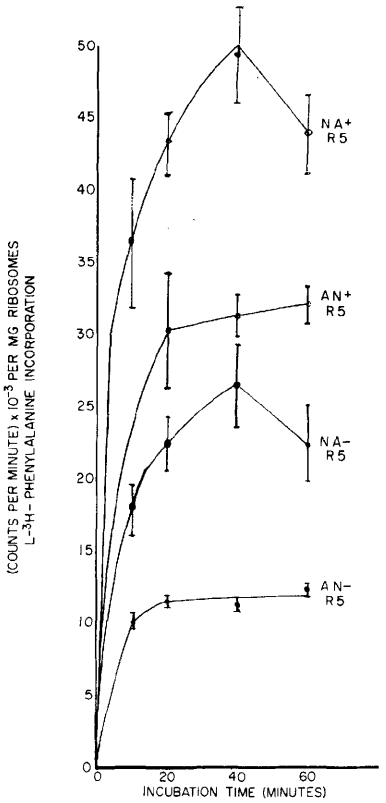

FIG. 2. Time-course of L-^{3}H-phenylalanine incorporation *in vitro* in the presence and absence of polyuridylic acid in crossing-over experiments with ribosomes and pH 5.1 fractions from normal and vitamin A-deficient animals. Protein synthesis assays were performed as described in Fig. 1. N_RA_5 = ribosomes from normal and pH 5.1 fraction from vitamin A-deficient animals. A_RN_5 = ribosomes from vitamin A-deficient animals and pH 5.1 fraction from normal animals. (+), presence of polyuridylic acid; (—), absence of polyuridylic acid.

Summary. Vitamin A deficiency enhances the *in vitro* capacity of rat liver ribosomes to make protein. This stimulatory effect of vitamin A deficiency on protein synthesis is due to the pH 5.1 enzyme fraction.

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