Vitamin A and Protein Metabolism

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The exact function of vitamin A outside the visual process is still unknown. Vitamin A is necessary for growth and therefore closely linked to protein metabolism. This paper reviews recent work on the relation between vitamin A and protein metabolism. Studies concerning the relationship between vitamin A and protein in human beings indicate that: vitamin A deficiency and protein malnutrition frequently occur together; the absorption of dietary vitamin A is impaired in acute protein malnutrition; the mobilization of vitamin A from the liver is hampered in protein–calorie malnutrition; dietary protein is required to mobilize liver reserves of vitamin A into the bloodstream; and increased protein intake results in greater vitamin A requirement. Animal studies have demonstrated that vitamin A influences: *in vivo* synthesis of serum proteins; synthesis of muscle protein *in vitro*; *in vitro* glycoprotein synthesis in the intestinal mucosa due to a factor in the pH 5 fraction; and the *in vitro* synthesis of glycoproteins in the cell membrane. The mechanisms whereby vitamin A influences protein metabolism remain unknown. It is not clear at this stage whether the effect of vitamin A on protein synthesis is direct or indirect.

Protein malnutrition and vitamin A deficiency are probably the two most common nutritional diseases in very large areas of the world today. In view of the widespread simultaneous occurrence of vitamin A deficiency and protein malnutrition in man, particularly in young children, several researchers have recently studied the possible relationship of protein and vitamin A metabolisms. Like all other essential nutrients, vitamin A is necessary for growth and therefore closely linked to protein metabolism.

Studies concerning the relationship between vitamin A and protein in human beings and animals will be reviewed here.

HUMAN STUDIES

Simultaneous Occurrence of Vitamin A Deficiency and Protein Malnutrition. The function of vitamin A outside of the visual process remains unknown. It is difficult to establish which biochemical systems are directly dependent on the vitamin because infectious diseases, inanition, and other forms of malnutrition usually complicate vitamin A deficiency in man and animals. Thus, children suffering from protein malnutrition frequently show signs of vitamin A deficiency (McLaren *et al.*, 1966), and xerophthalmia and keratomalacia are frequently accompanied by general malnutrition (Oomen, 1953).

In a classic paper on xerophthalmia in Danish infants, Bloch (1921) drew attention to the importance of various stresses, including intercurrent diseases not directly related to avitaminosis A, in precipitating the development of xerophthalmia. One form of stress appeared to be a diet containing excessive amounts of carbohydrates, now recognized as protein–calorie malnutrition or kwashiorkor.

The incidence of vitamin A deficiency in children suffering from protein–calorie malnutrition varies in different parts of the world. In Africa, children with protein–calorie malnutrition are less frequently and less severely deficient in vitamin A than children in Indonesia and India. Thus, in Durban, South Africa, Scragg and Rubridge (1960) reported that only 14 of 1565 children with kwashiorkor had keratomalacia. In Indonesia, Oomen (1954) observed keratomalacia and xerophthalmia in 29 of 44 children with advanced malnutrition. In India, reports of vitamin A deficiency in children with kwashiorkor are frequent; Achar and Benjamin (1951) found that 55 of 150 patients with kwashiorkor suffered from vitamin A deficiency. Venkatachalam and Gopalan (1960) and Chandra *et al.* (1960) reported that 32% of the children with kwashiorkor observed at Hyderabad and 36% of the kwashiorkor patients at Coonoor had signs of vitamin A deficiency. Poey (1957) observed that among the patients with kwashiorkor in the Pediatrics Department of the University Hospital in Djakarta, 49% also suffered from xerophthalmia, 10% had Bitot spots, and 20% had keratomalacia.

Absorption and Transport of Vitamin A in Protein Malnutrition. Frequently children suffering from kwashiorkor have low serum vitamin A levels (Arroyave *et al.*, 1959; Gopalan *et al.*, 1960; Trowell *et al.*, 1954).

In protein malnutrition, the absorption of dietary vitamin A from the intestinal tract is impaired. This has been demonstrated by Arroyave and his collaborators (1959) who gave large doses of vitamin A palmitate in oil to children with acute kwashiorkor. These patients did not show the marked increase in plasma vitamin A that occurs in well-nourished subjects under these circumstances. Roels and collaborators (1963) studied the effect of protein and fat supplements on vitamin A-deficient Indonesian children. Serum vitamin A levels in vitamin A-deficient boys receiving a protein supplement but no additional vitamin A rose rapidly to 40% above their starting levels by the end of the first week of treatment. By the end of the second week, however, the serum vitamin A level had fallen back to the initial levels, where it stayed throughout the supplementation period. During the first week of protein supplementation, more than the usual amount of vitamin A was mobilized from the liver reserves. When those reserves were depleted, the serum vitamin A level returned to that before supplementation; some equilibrium had been established between the total vitamin A intake and the serum vitamin A level.

Some interesting reports on the relation between protein malnutrition and vitamin A status have come from INCAP in Guatemala, where Arroyave *et al.* (1961) observed that, whereas all children with kwashiorkor had very low initial serum vitamin A levels, many of them showed a marked increase in serum vitamin A during the first 1–2 weeks of treatment even when receiving a diet containing no vitamin A. The increase in serum vitamin A paralleled the increase in serum protein concentration. Liver biopsies on four patients revealed that the increase in serum vitamin A occurred only when the liver still contained vitamin A reserves.

Adequate serum proteins or dietary proteins are therefore

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necessary to mobilize the vitamin from the liver into the bloodstream. Alternatively, the lowered or imbalanced protein intake, which gave rise to kwashiorkor, may have reduced the requirement for vitamin A and its mobilization from the liver. Arroyave and his collaborators did not find this rise in serum vitamin A when they gave skimmed milk treatment to kwashiorkor patients whose liver vitamin A reserves were very low to start with.

Recently it has been shown that retinol circulates in human plasma bound to a specific transport protein, retinol binding protein (RBP). The purified RBP has a molecular weight of 21,000. There seems to be one binding site for one molecule of retinol per molecule of RBP. In plasma, RBP circulates as a complex together with another larger protein with pre-albumin mobility. On electrophoresis, pre-albumin and RBP complex with each other in 1:1 molar ratio (Kanai *et al.*, 1968).

Protein Intake and Vitamin A Requirement. The implications of Arroyave's observations are important for the dietary protein supplementation of children in areas where there is a high incidence of kwashiorkor and where vitamin A status is marginal. Since the skimmed milk supplement, frequently given in such areas to combat protein malnutrition, will increase the vitamin A requirement, it may mobilize the last reserves of vitamin A from the liver and thus precipitate vitamin A deficiency. One should not interpret this as an argument against feeding a high-grade protein to children to prevent or cure kwashiorkor, but it should emphasize the need to supplement this high-grade protein with vitamin A. Oomen *et al.* (1964) report that there have been epidemic outbreaks of vitamin A deficiency coinciding with the distribution of skimmed milk by the United Nations International Children's Emergency Fund (UNICEF) in Brazil. This was attributed to the fact that vitamin A capsules, which were distributed with the milk, were not given to the children for various reasons—the children did not like them; the parents took the capsules and gave only the milk to the children; or the parents sold the capsules to make a little money. It is obvious that the milk could not have caused the eye lesions, whereas the lack of vitamin A might have done so. Parents were told that if they did not give the vitamin A capsules with the milk, their children might lose their sight, particularly when the milk was the only food they received. This obviously stresses the enormous importance of supplementing skim milk with vitamin A because increased protein intake causes increased vitamin A requirement. It was not until the fall of 1968 that nonfat dry milk used in the food donation programs in the United States was fortified with vitamin A.

Halder and Sundararajan (1958) demonstrated clearly that protein and vitamin A are both required for growth. They supplemented 500 children every day over a prolonged period of time with skimmed milk protein and a second group of 500 children with skimmed milk protein fortified with vitamin A. They found that the children who received the vitamin A-enriched skimmed milk supplement increased in height and weight much more rapidly than the children who received the skimmed milk supplement alone.

It is clear that in man: vitamin A deficiency and protein malnutrition frequently occur simultaneously; the absorption of dietary vitamin A is impaired in acute protein malnutrition; adequate serum and dietary proteins are necessary for the mobilization of vitamin A from the liver and its transport in the blood; increased protein intake results in greater vitamin A requirement; and both protein and vitamin A are required for growth.

ANIMAL STUDIES

Numerous animal studies were undertaken to elucidate the mechanism of the relation between vitamin A and protein metabolism, but so far they have not led to a clear unerstanding of this system.

Intestinal Conversion of Carotene to Vitamin A. Since carotenoids are the most important source of vitamin A for people suffering from protein malnutrition, an understanding of the effect of this deficiency on the utilization of dietary carotenoids is quite important. Deshmukh and Ganguly (1965) kept 50-g rats with low liver reserves of vitamin A on a diet containing 5, 10, and 20% casein for 30 days. One rat from each group was then given 3 mg of β-carotene dissolved in oil mixed with 1 g of fat-free diet. The β-carotene and total vitamin A contents of the stomach, intestinal contents, intestinal wall, blood, and liver of the rats were then determined at different time intervals ranging from 1 to 24 hr after the dose. The concentration of total vitamin A in the intestinal wall, blood, and liver was directly proportional to the protein intake, while the β-carotene value of the intestinal wall and intestinal contents were inversely related to the dietary protein levels. When similarly treated rats were given 4 mg of β-carotene daily for 7 consecutive days, their protein intake was directly proportional to the vitamin A content of their tissues and inversely proportional to their fecal carotene.

The intestinal conversion of β-carotene to vitamin A in the rat is therefore clearly related to protein intake.

Olsen (1961) and Olsen and Hayaishi (1965) have shown that retinal is an intermediate in the conversion of β-carotene to vitamin A. Deshmukh *et al.* (1964) have shown that during protein malnutrition in rats, the reduction step of retinal to retinol in the conversion process of β-carotene to vitamin A is most affected by protein deficiency.

Absorption and Storage of Vitamin A. Deshmukh *et al.* (1964) demonstrated that increasing dietary protein levels lead to more rapid intestinal absorption of retinyl esters in the rat resulting in a faster increase in the blood vitamin A concentration and more rapid liver storage of the vitamin. However, over longer time periods, all rats stored comparable amounts of vitamin A in their livers, irrespective of the protein level of their diets. Reduced protein intake slows down the absorption of vitamin A.

Storage and Mobilization of Vitamin A. Rechcigl *et al.* (1962) reported that when rats with adequate starting levels of liver vitamin A were fed different levels of dietary protein on a vitamin A-deficient diet, the depletion of liver vitamin A stores was directly proportional to the protein intake. More vitamin A was utilized by the organism as the dietary protein intake increased. These studies were done by administering large doses of vitamin A prior to the beginning of the experiment, thereby eliminating changes in the absorption of dietary vitamin A with different levels of the dietary protein. Esh and Bhattacharya (1960) demonstrated that prolonged inadequate or suboptimal protein feeding lowers liver storage of vitamin A in rats, implying a reduced absorption and transport of vitamin A. In a set of interesting experiments, Mahadevan *et al.* (1965) and Deshmukh *et al.* (1964) kept male rats, whose liver vitamin A reserves had previously been built up with a dose of 10 mg of retinyl acetate, for several weeks on diets containing 5, 10, and 20% casein. Their blood vitamin A concentrations were directly proportional to their blood albumin levels. Thus, blood vitamin A and albumin levels decreased progressively in the rats receiving the 10% casein diet, but the fall in blood vitamin A was much steeper in those animals receiving the 5% casein diet. When

the protein-depleted rats were fed the 20% diet, both albumin and vitamin A levels of blood returned steadily to the normal values. Control rats receiving 20% casein diet throughout had the least amount of vitamin A in their livers, while those receiving the lowest protein diet had the highest liver stores.

Vitamin A Enzyme Activity in Protein Deficiency. McLaren *et al.* (1966) have shown that in homogenates of rat pancreas and intestinal mucosa, the specific activity of retinal oxidase and of the enzymes catalyzing the hydrolysis and synthesis of retinyl esters is directly proportional to their dietary protein intake.

Vitamin A and Protein Synthesis. To test whether vitamin A deficiency influences protein metabolism directly or indirectly, Roels *et al.* (1964) studied the incorporation of ^{14}C-labeled amino acids into tissue proteins of vitamin A-deficient rats and pair-fed normal controls. When clear symptoms of vitamin A deficiency appeared, the animals were killed and their diaphragms removed. After rinsing the diaphragms with ice-cold Krebs–Henseleit buffer, they were incubated in fresh Krebs–Henseleit buffer for 2 hr at 37°, in the presence of ^{14}C-labeled amino acids. The rate of incorporation of uniformly labeled ^{14}C-phenylalanine and of methyl ^{14}C-labeled methionine was significantly higher in pair-fed controls. The mechanism whereby vitamin A deficiency increases the rate of incorporation of amino acids into muscle protein remains unknown.

de Luca *et al.* (1969) reported that vitamin A deficiency caused a marked decrease in the number of goblet cells in the small intestine of the rat but no other morphological changes were revealed by electron microscopy. *In vitro* incorporation of labeled amino acids into the proteins of the rough endoplasmic reticulum of the intestinal mucosa was reduced to less than one-half of the normal level in vitamin A deficiency. The lesion was located in the pH 5 fraction. These authors concluded that protein synthesis by membrane-bound but not by free polyribosomes of intestinal mucosa is depressed in vitamin A deficiency and that the vitamin is therefore involved, directly or indirectly, in protein synthesis at the translation level.

Secreted proteins are thought to be synthesized on membrane-bound polyribosomes. de Luca *et al.* (1970b) therefore determined whether the synthesis of any intestinal glycopeptide *in vivo* and *in vitro* would be affected by vitamin A. They found no difference in the 1-^{14}C-glucosamine incorporation into total glycoprotein in the intestinal mucosa from vitamin A-deficient and pair-fed normal control rats. Analysis of the glycoproteins by DEAE, Sephadex A, and Sepharos column chromatography showed that ^{14}C-D-glucosamine into a fucose-containing glycopeptide declined markedly. The glycopeptide was found to contain glucosamine, galactose, fucose, and sialic acid in the molar ratio 3:3:1:0.25. This glycopeptide could be a precursor of other glycopeptides when the product synthesis is interrupted by vitamin A deficiency.

In another study, de Luca *et al.* (1970a) demonstrated that a mannolipid synthesized by a membrane-rich fraction from rat liver in the presence of 15-^{14}C-retinol and GDP-mannose-^{14}C contained a polar metabolite of ^{14}C-retinol as well as ^{14}C-mannose. Retinol stimulated the *in vitro* incorporation of ^{14}C-mannose from GDP-mannose-^{14}C into the mannolipid and glycoproteins. These results suggest that vitamin A or one of its metabolites may function in mucus-secreting tissues by acting as a lipid intermediate, carrying mannose from GDP-mannose for the biosynthesis of the secreted glycoprotein.

CONCLUSION

Growth depression is one of the characteristic symptoms of vitamin A deficiency, and this disease occurs most frequently in the growing child or animal.

When growing young rats are depleted of vitamin A, their body weight gain is depressed early in the course of deficiency, before noticeable changes in food intake occur and without differences in linear growth. In addition, measurement of epithelial mitosis indicates that cell division is not reduced until a weight plateau and subsequent weight loss occur. In effect, the animal will eat and grow even though weight gain is less than normal. This inefficient weight gain appears related to a decreased utilization of dietary protein.

There is undoubtedly a very close relation between vitamin A and protein synthesis and between dietary protein intake and vitamin A utilization. It remains unknown, however, whether this relation is direct or indirect. Although many aspects of the metabolic functions of vitamin A have been studied and vitamin A has been synthesized and produced at a very low cost, vitamin A deficiency and protein malnutrition still are two most serious nutritional problems in the world today.

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