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PRESENT KNOWLEDGE OF VITAMIN A

Man's earliest knowledge of vitamin A was a deduction from symptoms of disease caused by its absence. Night blindness, a marked impairment of vision at low light intensity, has troubled man for thousands of years. It is undoubtedly for this reason that the visual process was of first interest in the study of vitamin A.

With the work of G. Wald (*Vitamins and Hormones* **18,** *417 (1960)*) and R. A. Morton (*Nature* **153,** *69 (1944)*) and others, much progress has been made in our understanding of this role of vitamin A. The retina of most vertebrates contains two distinct photoreceptor systems. The rods are especially sensitive to light of low intensity; the cones receive high light intensities and colors. Retinal is the prosthetic group of photosensitive pigments of the rods in land vertebrates and in marine fish, 3-dehydroretinal in fresh water fish.

The biochemical mechanism of cone vision is analogous to that of rod vision; photoreceptors of both systems contain identically the same chromophore—either retinal or 3-dehydro-retinal, depending upon the animal species. The protein moiety of these systems is not the same in rods as in cones, so that the major difference between the visual pigments in each is the protein bound to the chromophore.

Biochemical reactions involved in the oxidation of retinol to retinal, and stereochemical changes in the side chain of the vitamin A molecule which occur in the visual process, have been studied in detail. All-*trans* retinol is oxidized to all-*trans*-retinal; this compound isomerizes to the 11-*cis* form which, combined with opsin, forms rhodopsin. After absorbing light quanta the 11-*cis* isomer of retinal or of 3-dehydro-retinal is converted to the corresponding all-*trans* form.

Energy to operate this reaction against a potential gradient is supplied by the light quanta. This energy exchange causes potential differences, which produce a nervous excitation transmitted via the optic nerves to the brain, resulting in visual sensations. The potential difference caused by the breakdown of rhodopsin when it absorbs light can be measured by an electroretinograph. This measurement can then be used to assess the vitamin A status of animals and man.

Absorption, transport, and storage of vitamin A in mammals also have been studied extensively.

In most mammals, the product ultimately absorbed from the intestinal tract after feeding provitamins A is vitamin A itself. There is, however, a great deal of species specificity in the ability of different mammals to absorb dietary carotenoids. Man and the bovines can absorb both vitamin A and the carotenoids, and convert carotenoids with provitamin A activity to the vitamin. In contrast, the rat and the pig do not absorb significant amounts of carotenoid pigments. However, they have the ability to convert provitamins A to the vitamin. Although the small intestine is the most important organ involved, other tissues are also capable of carrying out this process. It has recently been demonstrated that perfusion of isolated rat liver with C^{14}-labeled beta-carotene produces labeled retinyl ester (*Nutrition Reviews* **21,** *238 (1963)*).

A number of factors affect absorption of the provitamins A from the intestine. The level of dietary fat is important in man's absorption of carotenoids. Fat represents less than 7 per cent of the total caloric intake in Ruanda and Urundi (Central

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Africa), and, although the diet there contains an ample supply of carotenoids, these are so inefficiently absorbed that vitamin A deficiency appears frequently in young boys. Small fat supplements markedly improve carotenoid absorption from the intestine in these boys, increase serum vitamin A levels, and alleviate symptoms of the deficiency.

Conjugated bile acids with one free hydroxyl group have a stimulating effect on carotene absorption and on cleavage of the carotene molecule in intestines of chicks, hamsters, rats, and rabbits. Low protein diets reduce intestinal absorption of Vitamin A and its esters.

Absorption and transport of vitamin A can be summarized as follows: dietary retinyl ester is hydrolyzed in the lumen of the intestine before passage across the mucosal cell wall. Retinol from dietary sources or resulting from the hydrolysis of dietary retinyl ester, passes the mucosal cell wall, and is reesterified inside the cell, preferentially with palmitic acid. Retinyl palmitate in chylomicrons travels through the lymphatic system, via the thoracic duct, to the blood stream, and is stored in the liver. Stored retinyl ester is hydrolyzed there by a liver enzyme; free retinol then travels via the blood stream to the tissues where a metabolic requirement exists. Retinol is mobilized from the liver and its level in blood is maintained, even on a diet without vitamin A, until all liver reserves are exhausted.

Like most other lipids, vitamin A and the provitamins A are transported in body fluids in the lipoprotein form. Retinyl ester occurs mainly in the Sf 10–100 lipoprotein fraction of the blood stream. Twenty per cent of the free retinol present in serum is associated with the Sf 3–9 serum lipoprotein fraction, which also carries about 80 per cent of the beta-carotene and lycopene in human serum. Since retinyl ester is transported from gut to blood stream in the chylomicrons (Sf > 400), the half life of the newly absorbed retinyl ester in chylomicrons must be short.

However, retinal, retinol, and retinyl ester are not the only active forms. All-*trans*-retinal has about 90 per cent as much activity outside the visual cycle as all-*trans*-retinyl-acetate. The bio-potency of retinal outside the visual cycle seems to depend entirely upon its enzymatic reduction to retinol: many animal tissues contain an enzyme capable of this reduction. In 1946, D. A. Van Dorp and J. F. Arens (*Rec. Trav. Chim.* **65,** *338 (1946)*) synthesized retinoic acid and demonstrated that this form can support growth of vitamin A deficient rats. When retinoic acid is fed to young rats on a diet lacking other forms of the vitamin, they grow normally but soon become night blind, eventually go completely blind, and cannot reproduce. It seems likely that retinoic acid is transformed into other compounds which may be "active" forms of vitamin A, and may play an essential role in animal metabolism.

A derivative of retinoic acid, possibly the glucuronide, has recently been discovered in the enterohepatic circulation of the rat. Synthesis of retinal epoxide with biological activity has also been reported.

The search for a general function of vitamin A outside the retina first centered on the possibility that it plays a role in keratinization, cornification, and mucus formation. It seems to be necessary for the formation of mucus secreting cells, which synthesize glycoproteins, and contain mucopolysaccharides. In vitamin A deficiency keratinization is more pronounced and formation of these cells is depressed.

Keratinization is required for formation of inert structural components of the body. Bone changes in vitamin A deficiency or in hypervitaminosis A were found to be associated with changes in chondroitin sulfate. All these observations point towards a possible role of the vitamin in mucoprotein synthesis and perhaps in synthesis of the mucopolysaccharide moiety of the muco-

protein molecule. It has been claimed that vitamin A deficiency reduces incorporation of sulfate into mucopolysaccharides, and that the step requiring vitamin A is the activation of sulfate to form 3′-phospho-adenosine-5′-phospho-sulfate.

An increased urinary excretion of inorganic sulfate has been observed in rats deficient in vitamin A. Sulfurylation of paranitrophenol by rat liver supernatant is also reduced in vitamin A deficiency. However, recent reports of markedly increased lysosomal sulfatase activity in livers of rats deficient in vitamin A, coupled with an increased release of acid hydrolases from the liver lysosomes, suggest that the apparent reduction in sulfate incorporation might be due to increased sulfatase activity. Vitamin A may well play a role in polysaccharide metabolism, but since synthesis and degradation occur simultaneously, the net result will depend on the relative rates of the two processes.

E. Mellanby's observation that hypervitaminosis A caused bone and nerve lesions in animals (*J. Physiol.* **101,** *408 (1942–1943)*) was followed by tissue culture studies, which indicated that addition of fairly large doses of vitamin A to the medium caused cessation of growth, followed by disintegration of the bone cartilage (*see Nutrition Reviews* **10,** *343 (1952)*). H. B. Fell and co-workers found later that an excess of vitamin A added to the medium dissolved the chrondroitin sulfate in cartilage. This effect was shown to be due to rupture of lysosomal membranes, followed by release of acid hydrolases from lysosomes into the medium. These hydrolases then caused tissue disintegration (*see Nutrition Reviews* **20,** *161 (1962)*).

The same effect has been observed in different species, and recent work has shown that liver lysosomes of rats deficient in vitamin A also become very labile. Normal vitamin A concentration ensures optimum stability, but large doses labilize the lysosomal membrane *in vivo,* as well as *in vitro.* This effect plays a role in many different membrane systems, such as the erythrocyte outer membrane and the mitochondrial membrane.

In carbohydrate metabolism, it was found that vitamin A deficiency did not disturb the tricarboxylic acid cycle. However, glycogen biosynthesis from acetate, lactate, and glycerol appears to be slowed down and can be reversed by cortisone administration.

Interaction between the vitamin A group of compounds and other members of the lipid class has also been studied extensively. Vitamin A metabolism is linked with that of coenzyme Q, vitamin E, vitamin D, the sterols, and the biosynthesis of squalene. Interaction of vitamins A and E seems to be important in regulating stability of biological membranes (O. A. Roels, M. Trout, and A. Guha, *Biochem. J.* **97,** *353 (1965)*). Morton and his collaborators found that ubiquinone (coenzyme Q) increased in the liver of vitamin A deficient rats (*see Nutrition Reviews* **19,** *218 (1961)*). Vitamin A deficiency increases synthesis of squalene and ubiquinone in rat liver and reduces cholesterol synthesis.

It has been shown that utilization of liver vitamin A stores is directly proportional to protein intake when animals are fed a diet low in the vitamin. Low protein diets retard the onset of deficiency symptoms.

Several studies have indicated that vitamin A influences synthesis of both serum and muscle proteins. Whether this effect is direct or indirect remains to be seen.

Protein malnutrition and vitamin A deficiency are probably the two most common nutritional deficiency diseases in the world today. Frequently both occur simultaneously, often with fatal results.

Patients with kwashiorkor often have very low serum vitamin A levels. When adequate dietary proteins are given, levels rise without administration of vitamin A, provided there are sufficient liver reserves. The implications are of the utmost impor-

tance to the nutrition of children in areas where there is a high incidence of kwashiorkor and vitamin A status is marginal. Since a skimmed milk supplement is frequently used in kwashiorkor treatment, and results in an increased vitamin A requirement, it may mobilize the last reserves from the liver, and thus precipitate vitamin A deficiency. This is not an argument against feeding a high grade protein to children with kwashiorkor, but emphasizes the need for a vitamin A supplement as well.

An excess of vitamin A may also have a very serious effect on man and animals. The acute form of vitamin A intoxication has been noted in persons ingesting excessively high single doses, or large quantities of polar bear liver. Chronic hypervitaminosis A occurs in patients who receive large doses for dermatologic conditions and continue subsequent intake without medical supervision. Severe cases of hypervitaminosis A have been encountered in faddists who include excessively large doses of vitamins in their daily diet.

A wide variety of signs and symptoms may appear. Fatigue, malaise, and lethargy are common complaints, frequently accompanied by one or more of the following symptoms: abdominal discomfort, bone and/or joint pain, severe throbbing headaches, insomnia and restlessness, night sweats, loss of body hair, brittle nails. Other symptoms may be exophthalmus, peripheral edema, yellow waxy pigmentation of the soles, palms, and nasolabial folds due to carotenoid deposits. Increased intracranial pressure and increased cerebral spinal fluid pressure have been observed. The latter symptom has been described frequently in bovines suffering from hypervitaminosis A.

The most serious public health problem related to this vitamin, however, is still its deficiency. The seriousness and clinical consequences, mainly in young boys throughout the world, have been emphasized in a recently published report (H. A. Oomen, D. S. McLaren, and U. Escapini, *Trop. Geograph. Med.* **16,** *271 (1964)*). The authors conclude that xerophthalmia remains a major health problem in many parts of the world, especially in rapidly growing urban centers of the East like Hong Kong, Djakarta, Manila, Saigon, and Dacca.

Xerophthalmia is a major cause of blindness in childhood, yet vitamin A deficiency is often the result of ignorance. Rich sources of provitamin A are frequently available at little or no cost to families in areas where the deficiency is prevalent. Therefore, nutrition education can play an important role in eradicating this disease. The protective effect of a large depot dose of vitamin A should be investigated. It is possible to administer a single large dose intramuscularly, intravenously, or orally, and obtain good liver storage. This protects the child for a relatively long period from the disastrous results of an inadequate dietary supply.

Vitamin A palmitate now sells for approximately five U.S. cents for 1,000,000 I. U. Since the daily requirement of an adult human is about 3,000 I. U., it is apparent that to provide the entire annual requirement of one human being would cost about five U.S. cents. It is truly amazing, in view of this, that vitamin A deficiency remains one of the most widespread forms of human malnutrition, with its untold consequences of social and individual misery.

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