2. Occurrence, Production, Use, Analysis and Human Exposure

2.1 Occurrence

The primary source of vitamin A is the precursor compounds, the carotenoids. There are more than 600 naturally occurring carotenoids, but only 50–60 of them possess vitamin A activity, producing at least one intact molecule of retinol or retinoic acid when metabolized, and only about 10 of them have nutritional relevance (Pfander, 1987).

The second important dietary source of vitamin A is retinyl esters, which are found in foods of animal origin. Retinyl esters are derivatives of retinol with fatty acids. The most important dietary retinyl ester is retinyl palmitate, with smaller amounts of retinyl oleate and retinyl stearate (Ong, 1994).

In several developing countries, synthetic vitamin A, often in the form of palmityl ester, is added to commonly consumed foods. Monosodium glutamate has been used as a vehicle in Indonesia (Muhilal *et al.*, 1988) and the Philippines, while vitamin A is added to sugar in several central American countries (Mejia & Arroyave, 1982), and ghee is used in Pakistan. However, while food fortification may be a short-term measure to cope with inadequacy of dietary intake, more sustainable measures must involve promoting the consumption of locally grown fruit and vegetables and improving social conditions.

In the developed world, synthetic vitamin A and carotenoids are added to a small number of foods such as dairy products and are also found in pharmaceuticals and cosmetics for oncological and dermatological use. β-Carotene and other carotenoids are also used extensively as food colourants (IARC, 1998).

2.2 Production

Retinol can be synthesized chemically. Early developments have been described by Moore (1957) and more recent approaches by Frickel (1984) and by Dawson & Hobbs (1994). Several pharmaceutical companies supply retinol with 99% purity. Provitamin A carotenoids may be converted to vitamin A by central cleavage to yield one or two molecules of retinal. This conversion is catalysed by a 15,15'-dioxygenase enzyme that is found in the intestinal mucosa, liver and other tissues (Devery & Milborrow, 1994; Nagao *et al.*, 1996). Retinal is then reduced by an aldehyde reductase to retinol. Eccentric cleavage of carotenoids yields β-apocarotenals with different chain lengths, which then may be shortened by β-oxidation to retinol. In addition, there is evidence that retinoic acid may be formed directly from such carotenoids as β-carotene by a still undefined pathway (Wang *et al.*, 1992).

Preformed vitamin A is found in the diet primarily in the form of retinyl esters. Retinol is formed in the intestine by hydrolysis of the long-chain retinyl esters catalysed by a brush border retinyl hydrolase and a non-specific pancreatic hydrolase (Blaner & Olson, 1994) (see Section 3.2.2).

2.3 Use and application

Vitamin A is an essential micronutrient for animals and man. Humans obtain their dietary supply from foods of both animal and plant origin, but the more dependent they are on plant sources, the more difficult it is to meet metabolic needs. Dietary requirements for vitamin A have been investigated by many

workers and their findings have been reviewed in various reports and by both national and international bodies (Olson, 1987; FAO/WHO, 1988; National Research Council, 1989). There are some wide differences between countries in the amounts of vitamin A considered essential to meet dietary requirements (McLaren, 1994), but most countries have generally adopted the arguments discussed in the FAO/WHO (1988) handbook. Requirements for infants are generally arrived at from the amount of vitamin A in breast milk (340–400 mg/day) and as children get progressively older, this amount is increased to allow for growth. In adults, requirements are based on the catabolism of total body vitamin A, with allowances for body size and storage. These calculations give figures of 700 mg/day for men and 600 mg/day for women. In addition, about 400 mg/day must be added during lactation. Despite the abundance of carotenoids in vegetables and fruits, more than 200 million children worldwide suffer from various degrees of vitamin A deficiency. Therefore, vitamin A is used as a supplement or in fortification of a large number of processed foods. In terms of the degree of public health importance of vitamin A deficiency, the World Health Organization (WHO) categorizes countries into those that have clinical or subclinical vitamin A deficiency and those that have no vitamin A deficiency-related problems (McLaren & Frigg, 1997). The group with subclinical problems is further subclassified as having severe, moderate or mild vitamin A deficiency, based on prevalence of low serum retinol levels in children (WHO, 1996). Vitamin A- and retinol-deficient states such as xerophthalmia and night-blindness can be reversed by treatment with vitamin A (retinyl acetate or palmitate) from either animal or synthetic sources (Tee, 1992). In subclinically vitamin A-deficient populations, supplementation during measles infection will reduce mortality, morbidity and the length of hospital stay (Barclay *et al.*, 1987; Hussey & Klein, 1990). The WHO, United Nations Children's Fund (UNICEF), the United States Agency for International Development, and many other national and non-governmental organizations aim to eliminate vitamin A deficiency as a public health problem in all countries by the year 2000 through a combination of supplementation, food fortification and nutrition education.

The following dosages are recommended by WHO and UNICEF for alleviating vitamin A-deficiency disorders in children in areas of high risk (McLaren & Frigg, 1997).

Subject	Oral dose (IU)
Children 1–6 years	200 000 every 3–6 months
Infants 6–11 months	100 000 every 3–6 months
Lactating mothers	200 000 once (within 3 months of delivery)

In cases where xerophthalmia has been diagnosed or in children with measles in marginally vitamin A-deficient populations, the supplemention would be as shown below.

Time of administration after diagnosis	Dose (IU)	
	< 1 year old	> 1 year old
Immediately	100 000	200 000
Next day	100 000	200 000
2–4 weeks later	100 000	200 000

The well documented effects of retinoic acid and synthetic retinoids on several dermatological disorders (for review, see Vahlquist, 1994) led to the development of commercial ointments containing retinyl esters. Several prescription drugs containing retinoic acid and its 13-*cis*-isomer are available for treating acne and photo-ageing skin. Vitamin A is also used in topical non-prescription cosmetics for improvement of appearance of wrinkles. The effect of these topical preparations is limited to the skin and does not contribute substantially to the vitamin A status of the body.

2.4 Human exposure

Preformed vitamin A in the diet is obtained from vertebrate animal sources rich in retinyl esters (tissues such as liver, kidney, dairy products and eggs). Liver and fish oils are the richest dietary sources of preformed vitamin A and

concern has been expressed by some authorities about potentially teratogenic effects of liver consumption during pregnancy (Eckhoff & Nau, 1990b). However, for many populations worldwide, these foods are used only sparingly. Hence, plant provitamin A carotenoids are usually the major food source of vitamin A. Unfortunately, poor bioavailability of carotenoids in vegetables restricts their usefulness, because the cellulose walls of plant cells are not easily broken down (de Pee & West, 1996). In contrast, carotenoids in fruits are more bioavailable. Fruits such as mangoes and pawpaw are good sources of vitamin A, but their consumption may be restricted to short seasonal periods. Volume 2 of the *IARC Handbooks of Cancer Prevention* treats this subject in depth. The average daily dietary intake of vitamin A in the United States is about 2.25 mg of retinol equivalents, of which 47% is derived from fruits and vegetables; 27% from fats, oils and dairy products and 27% from meat, fish and eggs. Provitamin A carotenoids provide about 50% of this intake (Tee, 1992).

The vitamin A activity of β-carotene and other provitamin A carotenoids has been arbitrarily defined as one-sixth and one-twelfth of that of retinol. Calculation of vitamin A intakes is generally satisfactory if retinol or β-carotene is in the main food constituents, but this is often not the case. Calculations of vitamin A intakes are made more difficult by variability in the way data are presented in food composition tables, some of which combine provitamin A carotenoids and retinol as vitamin A, while others separate them (Tee, 199).

The dietary intake of vitamin A in different populations in the world varies greatly. In addition, intake data outside the industrialized world are incomplete, particularly where vitamin A deficiency is a problem. The incompleteness of food composition tables for carotenoids in foods also makes accurate measurements of total vitamin A intake difficult and complicates the design of supplementation programmes (McLaren & Frigg, 1997). Substantial amounts of vitamin A may also be taken as single or multivitamin supplements. These usually contain vitamin A in the range of 3000 to 10 000 IU. In a study by Willett *et al.* (1983), supplementation of well nourished adults with β-carotene (30 mg daily for 16 weeks) increased the plasma levels of β-carotene but not the plasma retinol levels. Also, a daily retinyl ester supplement (25 000 IU) did not increase the plasma retinol levels, illustrating the fact that plasma retinol concentration is under homeostatic control (see Section 3.1).

2.5 Analysis

Measurement of plasma retinol is probably the most common method used to assess vitamin A status. As mentioned above however, the concentration of plasma retinol is homeostatically controlled over most of the range found in human subjects, so that interpreting status from low plasma values can be difficult.

In many developing countries, plasma retinol levels are lower than those seen in the western world, particularly in environments where viral and bacterial infections and parasite infestations are common (Thurnham, 1997). The WHO (1996) has suggested that serum levels of ≤0.7 mmol/L indicate a high risk of vitamin A deficiency. There is evidence that concurrent infection lowers concentrations of circulating retinol by suppressing the synthesis of retinol-binding protein (RBP) in the liver (Rosales & Ross, 1996). Frequent infections also contribute to poor vitamin A status by reducing intake and increasing the potential excretion of retinol in urine (Stephensen *et al.*, 1994).

The concentration of plasma carotenoids also gives information on vitamin A status. The concentration of plasma β-carotene depends on dietary intake and availability of preformed vitamin A in the diet. Based on currently available data, the higher the vitamin A intake, the lower the conversion of provitamin A carotenoids to retinol. Levels of non-provitamin A carotenoids like lutein also provide information on vegetable intake. The presence of lutein in the plasma indicates that green vegetables are consumed, implying that β-carotene, even if undetectable in the plasma (Thurnham *et al.*, 1997), is present in the diet. It is therefore advisable to measure several markers of vitamin A status and some of the more useful methods currently being used are described below. In the

following descriptions, the terms plasma and serum are used interchangeably.

2.5.1 Functional methods

2.5.1.1 Night-blindness

This method of assessment involves an ophthalmological instrument, the dark adaptometer, which measures the speed of adaptation of the eye to low light conditions. However, the method is not suitable for field conditions and night-blindness is usually assessed by interviews of the patients and guardians as well as careful observation of the ability of children to see in dim light (Sommer *et al.*, 1980). This method is suitable for the assessment of early signs of moderate to severe vitamin A deficiency. At the community level, it is useful to enquire if there is a local word for night-blindness. A positive response is highly indicative that the community has or recently had a vitamin A-deficiency problem.

2.5.1.2 Conjunctival impression cytology *(Wittpenn et al., 1986)*

This method involves assessing the morphological appearance of cells from the eye epithelium obtained by pressing a small piece of filter paper onto the conjunctiva. Normally, conjunctival cells resemble epithelial cells and the number of mucin-producing goblet cells is high. In the vitamin A-deficient state, the conjunctival epithelium becomes flattened in appearance and the number of goblet cells is dramatically reduced (WHO, 1996). It has been suggested that this method should be the one for assessing marginal vitamin A deficiency in children (Olson, 1994a). Unfortunately, it is labour-intensive, assessment is dependent on individual skills and the two-month delay for the reading to normalize following treatment of children who previously had low liver reserves of vitamin A and plasma retinol (< 0.7 mol/L) reduces its efficiency in monitoring changes in status (Amédée-Manesme *et al.*, 1988).

2.5.2 Biochemical methods

2.5.2.1 Relative dose response (RDR) *(Underwood, 1990a,b)*

This method is based on release of apo-RBP (i.e. unbound protein, as opposed to holo-RBP, the retinol-bound protein) from the liver to the serum. In individuals with low vitamin A status, apo-RBP accumulates in the liver. Some apo-RBP is released after administration of an oral dose of vitamin A (for example, as retinyl acetate in oil). First, baseline serum retinol (A_0) in a blood sample is measured by high-performance liquid chromatography (HPLC). Five hours after an oral dose of 450–1000 µg retinyl acetate, a second sample is analysed for serum retinol (A_5). An RDR value is calculated from $RDR = (A_5 - A_0) \times 100/A_5$. A value over 20% is a positive indication of vitamin A inadequacy, which indicates a low vitamin A concentration in the liver (≤ 0.07 µmol/g vitamin A) (McLaren & Frigg, 1997).

2.5.2.2 Modified relative dose response (MRDR) *(Tanumihardjo et al., 1990a)*

This method is based on the administration of a single oral dose of 0.53 mmol of 3,4-didehydroretinyl acetate and subsequent analysis of a blood sample 4–6 h later for both 3,4-didehydroretinol and retinol. Vitamin A status is determined by the ratio between 3,4-didehydroretinol and retinol as follows:

MRDR = 3,4-didehydroretinol/retinol

MRDR is considered to be normal when the ratio is below 0.06 (McLaren & Frigg, 1997).

At a population level, the WHO has classified countries as mildly, moderately or severely deficient, based on a prevalence of vitamin A deficiency, as measured by any of these three tests, of < 20%, > 20–30% and > 30%, respectively.

2.5.2.3 Serum retinol

Serum concentrations of retinol can be measured directly by HPLC of an organic solvent extract of a serum sample using reversed-phase chromatography on a C_{18} column and detection by UV absorption at 326 nm (Frolik & Olson, 1984; Catignani & Bieri, 1983; Barua *et al.*, 1993). Since retinol concentrations in serum are under homeostatic control, the values are useful indicators of status only when concentrations are very high or very low. Measurements of serum retinol are most useful

at the population level for the assessment of intervention programmes in vitamin A-deficient populations. The concentration of retinol in well nourished adults is 1.8–3.2 μmol/L. The median and range (5–95%) are lower in women (e.g., 1.8 μmol/L; 1.2–2.8 μmol/L) than in men (2.2; 1.4–3.2) (Gregory *et al.*, 1990). The WHO suggests that a serum retinol level of < 0.7 μmol/L in 10% of a population is indicative of a public health problem (WHO, 1996).

The validity of measurements of plasma components is critically dependent on stability during storage. In general, the stability of retinol and its esters is very good when plasma or serum samples are stored in sealed containers at −70 °C. Carotenoids are somewhat less stable than retinoids under similar storage conditions. The stability of retinyl esters in lipoproteins has not been carefully studied. With regard to short-term storage, when serum was left stored overnight at 4°C in the dark, retinol and α-tocopherol concentrations were unchanged, whereas carotenoid concentrations decreased by an average of 5% (Key *et al.*, 1996). To maximize stability for long-term storage at −70°C, serum samples should be prepared as soon as conveniently possible after blood is drawn. The gas space above the sample should be kept as small as possible, and the tube should be hermetically sealed. Degassing the samples before storage is helpful, and filling the gas space, preferably with argon or with oxygen-free nitrogen, is recommended but not essential. Several aliquots of each sample should be prepared to avoid the adverse effects of repeated freezing and thawing at a later time.

2.5.2.4 Vitamin A in food

Determinations of native or supplemental vitamin A in food in the form of provitamin A carotene and retinyl esters are usually performed by reversed-phase high-performance liquid chromatography on a C_{18} column. Foods may or may not have been saponified before the preparation of an organic extract. β-Carotene and other carotenoids are detected by UV absorption at 450 nm and retinol and its esters at 326 nm (Furr *et al.*, 1992). Data in food composition tables on food analyses of retinol and provitamin A carotenoids should be interpreted with care. Values for preformed vitamin A, the form found in foods of animal origin, are usually more accurate than data on carotenoids in vegetable foods. In addition, preformed vitamin A is better and more predictably absorbed than provitamin A sources. Absorption of the latter can vary appreciably depending on food preparation and cooking methods: chopping, heating and the presence of oil tend to increase bioavailability. The methodology used to assess vitamin A status using dietary enquiry methods and food composition tables is described in Section 4.

2.5.2.5 Milk analysis

Human milk contains retinyl esters and provitamin A carotenoids (Canfield *et al.*, 1997; Khachik *et al.*, 1997) in amounts which reflect the vitamin A status of the mother. Therefore, milk analysis provides a non-invasive method of assessing vitamin A status. This analysis includes a saponification step before organic extraction. The extract is then analysed as for serum analysis. A retinol concentration below 1.05 μmol/L in milk is considered by the WHO to reflect vitamin A deficiency and a public health problem is considered to occur when more than 25% of the lactating female population has lower milk levels (McLaren & Frigg, 1997).