The effect of vitamin A on epithelial integrity

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Vitamin A is the generic term for a variety of fat-soluble substances including retinol, retinyl palmitate and the provitamin A carotenoids such as all-*trans*-β-carotene. Vitamin A is commonly known as the anti-infective vitamin and has an essential role in vision and cellular differentiation, the latter providing a unique core mechanism helping to explain the influence of vitamin A on epithelial barriers. Alterations in the epithelial lining of vital organs occur early in deficiency, suggesting a potentially important role for the barrier function. Vitamin A deficiency (VAD) is most commonly recognized in the eye. The conjunctival-impression cytology test detects the presence of larger irregular keratinized cells and the absence of mucous-secreting goblet cells, indicative of VAD. The method is simple, quick and sensitive in populations where VAD is present. In the respiratory tract, observational studies all show an association with VAD, although vitamin A supplementation studies appear to have little effect on respiratory disease. Organ-specific targeting may improve success rates. The dual-sugar intestinal-permeability test allows the effect of vitamin A supplementation to be monitored on the gastrointestinal tract. Two vitamin A supplementation studies were carried out recently in Orissa State, India. Healthy infants of weaning age were administered orally eight weekly doses of 5·0 mg retinol equivalents and hospitalized infants received one large oral dose 60 mg retinol equivalents in the form of retinyl palmitate. Improvements in gut integrity and haematological status were observed in both studies. In summary, the response of the eye to vitamin A supplementation is well established; the present review highlights some of the more recent observations examining the effects of vitamin A.

Vitamin A: Conjunctival-impression cytology test: Dual-sugar intestinal-permeability test

‘*Vitamin A builds fences that keep germs out.’ E.V. McCollum 1935*

‘Vitamin A’ is a generic term for a variety of related compounds, including retinol, retinyl palmitate, retinoic acid and the pro-vitamin A carotenoids. The fat-soluble substance is usually found in animal and dairy products as the ester, retinyl palmitate. The alcohol retinol is formed during hydrolysis in the small intestine where it is taken up actively by the enterocytes. The most active provitamin A carotenoid is all-*trans*-β-carotene, which is present in yellow–orange fruits and dark-green leafy vegetables. Intestinal cells convert provitamin A carotenoids to retinaldehyde, the majority of which is reduced to retinol. Bioavailability of provitamin A carotenes varies widely, depending on how the food is prepared and consumed.

Vitamin A is commonly known as the anti-infective vitamin. At the beginning of the twentieth century, animals fed on ‘pure’ diets containing only lard and carbohydrates with no vitamins ceased to grow, lost weight and became susceptible to infections. Administration of ‘accessory factors’ found in dairy products and cod-liver oil reversed the process. McCollum (McCollum & Davis, 1915) termed the critical factor ‘Fat-soluble A’. By 1920 the clinical manifestation of vitamin A deficiency (VAD), and its cause and cure were well established.

Vitamin A has an essential role in vision and cellular differentiation, the latter being particularly important in growth, reproduction and immune response, and in maintaining epithelial integrity. The critical function vitamin A plays in regulating cellular differentiation provides a unique ‘core’ mechanism that would at least partly explain its influence on epithelial barriers, immune competence, healing, resistance and recovery (Ross, 1992). No nutritional deficiency is more synergistic with infection than vitamin A. The two main mechanisms involved in the prevention of disease are the effect of vitamin A on the immune system

Abbreviations: CIC, conjunctival-impression cytology; L, lactulose; M, mannitol; VAD, vitamin A deficiency.

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and the effect on epithelial integrity, the subject of the present review. All epithelial surfaces, including the eye, skin, trachea, salivary gland, vaginal epithelium and gastrointestinal tract, are involved. Alterations in the epithelial lining of vital organs occur early in VAD, suggesting a potentially important role for the 'barrier function'. Tissue integrity is vitally important for protection against environmental pathogenic organisms.

Epithelial tissues

Eye

VAD is most commonly recognized in the eye. Night-blindness and its successful treatment with animal liver was known to the ancient Egyptians at least 3500 years ago (Wolf, 1978), and was mentioned by Hippocrates. Xerophthalmia (from the Greek: xero, dry; ophthalmia, inflamed eye) is a constellation of ocular manifestations long associated with VAD, representing the 'classical' presentation of this particular form of malnutrition. Deficiency symptoms are progressive from night-blindness, conjunctival xerosis, Bitot's spots and corneal xerosis to corneal ulceration (keratomalacia), corneal scar and xerophthalmia fundus (World Health Organization, 1995).

Vitamin A status can be assessed by functional tests that measure epithelial integrity. Keratinizing metaplasia is a potentially practical index, and as early as 1925 the scrapings of cornea, nose, mouth and vagina were recommended as a diagnostic tool of VAD (Wolbach & Howe, 1925). The early studies were neither qualitative nor validated against any other index of vitamin A status. More detailed knowledge about the normal and deficient nature of conjunctival epithelium, particularly the geographic distribution of goblet cells (Kessing, 1968) and their response to vitamin A deficiency (Sommer *et al*. 1982), created renewed interest in the approach. The system of 'simple conjunctival biopsy' was described by Egbert *et al*. (1971), and Nelson *et al*. (1988). Cellulose acetate filter paper was briefly applied to the conjunctival surface to remove the superficial layers of the conjunctival epithelium. The biopsy technique, renamed conjunctival-impression cytology (CIC) provides evidence of keratinizing metaplasia. Vitamin A-unresponsive and -responsive Bitot's spots have an identical biological appearance; however, the CIC test detects the absence of mucous-secreting goblet cells and the presence of larger irregular keratinized cells. Initial trials demonstrated markedly abnormal CIC from children with mild xerophthalmia when compared with normal children or following vitamin A treatment (Wittpenn *et al*. 1986). There was a close correlation between the prevalence of abnormal CIC and the severity of deficiency suggested by three other indicators of vitamin A status (serum retinol, relative dose response and modified relative dose response). Of the children with clinically-responsive xerophthalmia and low vitamin A levels 93% had abnormal cytology, and 6% of children with normal eyes and serum retinol levels between 0·09 and 0·10μmol/l (250–290 μg/l) had abnormal CIC (Natadiastra *et al*. 1988). The validity of CIC has been further confirmed by the following: comparison of liver retinol concentrations and relative dose response in French children with normal eyes but with hepatic disease (Amedee-Manesme *et al*. 1988); comparisons of abnormal CIC rates in xerophthalmic and non-xerophthalmic children in India (Reddy *et al*. 1989); correlation of CIC with serum and breast-milk retinol in Indonesian women (Miller *et al*. 1993) and by correlations of CIC with other indices of vitamin A status (Tanumihardjo & Olsen, 1991).

CIC is of particular advantage under field conditions as it does not require sophisticated laboratory analysis, storage or transport. However, there are problems of compliance with small children, who can be frightened of the paper being set on their eye. CIC is not without its limitations and several reports (for example, see Gadomski *et al*. 1989) have questioned its validity and/or interpretation. In theory, although not always in practice, and ideal marker would detect subclinical deficiency, when stores are below 'safe' levels. By definition, a functional abnormality is evidence that physiologically-significant deficiency already exists. Hence, the CIC marker has been more successfully used in epidemiological studies than as a diagnostic tool for individuals, especially in areas of marginal deficiency (Natadiastra *et al*. 1988). When using CIC Coutsoudis & Way (1996) noted that allergic conjunctivitis could be mistaken for VAD in South African children.

Respiratory tract

Observational studies (see Vijayaraghavan *et al*. 1990) all show an association between VAD and respiratory infection. However, a meta-analysis (Bates, 1995) showed a lack of impact of vitamin A supplementation, although there were some indications of reduced severity and possibly reduced duration of respiratory symptoms. Interestingly, some researchers suggest that increased cough following supplementation may indicate greater mucus production and removal of infective material (Herrera *et al*. 1996). A promising method of treating respiratory infections using organ-specific targeting was suggested by Biesalski (1996). In rats direct inhalation of 0·9 mg retinol equivalents as retinyl esters, produced an increase in plasma retinyl ester from 0·16μmol/l (45 μg/l) to 0·66μmol/l (190 μg/l) with no side-effects.

It was suggested by Shenai *et al*. (1987) that vitamin A supplementation in preterm infants was of benefit in bronchopulmonary dysplasia. It was reported by Coutsoudis *et al*. (1993) that initial VAD in preterm infants does not predispose them to respiratory distress syndrome or pneumonia, but by 3 months post-partum vitamin A status was adequate and similar to that of full-term infants.

Gastrointestinal tract

Until recently, mucosal damage could only be assessed by endoscopy and/or biopsy of the small intestine. Using such techniques a number of studies have shown a substantial reduction in the number of goblet cells per duodenal villus when compared with control and pair-fed mice (Ahmed *et al*. 1990), a reduction in luminal mucus (Meneghetti *et al*. 1989), and decreased cellular division preceding histological abnormalities in mildly-VAD animals (Zile *et al*. 1981).

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Non-invasive techniques (Lunn *et al*. 1989; Northrop *et al*. 1990) have been introduced which estimate specific aspects of mucosal function and integrity and allow repeated measurements to be carried out, even under field conditions. Absorption across healthy intestinal mucosa is highly selective. 'Inert molecules' pass across the absorptive surfaces at rates determined by concentration gradient, surface area and time, as well as the permeability of the intestinal mucosa. The mucosa demonstrates marked discrimination with respect to the molecular dimension and solubility of inert molecules, this discrimination becoming impaired with mucosal damage (Menzies *et al*. 1983). The xylose-absorption test has been used as a preliminary screening procedure for gastrointestinal integrity (Hubble & Littlejohn, 1963), but has limitations. Gastric emptying, intestinal transit, blood flow and renal function variables reduce the correlation between xylose absorption or excretion and the degree of mucosal damage (Lamabadusuriya *et al*. 1975). Dual-sugar absorption tests are not affected to the same extent by such limitations. A variety of markers have been utilized to indicate gastrointestinal integrity, e.g. ingestion of lactulose and rhamnose showed that during kwashiorkor, abnormal intestinal permeability correlated with disease severity and improved only slowly with nutritional rehabilitation (Brewster *et al*. 1997). Elia *et al*. (1987) investigated the factors affecting the intestinal uptake and urinary excretion of mannitol (M), lactulose (L) and Cr-EDTA in normal subjects and three patients with ileostomy. They suggested that the uptake of L is slower than that of M, and Cr-EDTA is readily absorbed in the colon. L and M are two non-metabolizable sugars that cross the mucosa paracellularly and transcellularly respectively. The ratio L : M in blood is an indicator of mucosal damage, L : M greater than 0·02 being considered abnormal. L : M values discriminated well between subjects with normal biopsy and those with villous atrophy. Fleming *et al*. (1996) successfully measured L and M by HPLC in serum 90 min after intake, while other workers have used automated enzymic assays (Lunn *et al*. 1989; Northrop *et al*. 1990). The HPLC results agree well with L : M values determined enzymically following a 5 h urine collection (r 0·88, $P = 0·05$), hence the two methods can be used interchangeably.

Northrop-Clewes *et al*. (1997) investigated the role of vitamin A on epithelial integrity using the L : M dual-sugar intestinal-permeability test. This test uses a weight-dependent dose of the two sugars (L and M) which are given to the infant dissolved in water. A urine bag is used to collect the urine produced during the following 5 h. The sugars are measured enzymically and the L : M value is an indicator of epithelial integrity. A healthy mucosal surface has villi which are long finger-like projections allowing maximal absorption of M, while L, the larger molecule, can only pass between the cells. Absorption of L is therefore minimal, resulting in a low L : M value. Damage to villi could include either a reduced surface area, e.g. by a flattening of the surface area, and/or damage caused by parasitic infestation (e.g. hookworms) causing leakiness of the gastrointestinal tract. In both situations the absorption of L increases relative to that of M, resulting in an elevated L : M value.

Faltering growth, infections, diarrhoea and anaemia remain major problems in the developing world. Retinol and Fe are transported in the bloodstream by negatively-charged acute-phase proteins which decrease during infection, hence retinol and Fe are 'trapped' in the liver and spleen during disease. Foods are frequently contaminated with pathogens, thus exposure to infections is increased in developing countries, and the risk of infection may be exacerbated by poor gastrointestinal integrity. Impaired gastrointestinal integrity may facilitate the translocation of bacteria across the mucosa. Studies carried out in rural Gambian infants showed evidence of impaired intestinal integrity and elevated acute-phase response, which accounted for up to 50 % of the growth faltering noted during the first 2 years of life (Northrop-Clewes *et al*. 1997). Seasonal data from the same Gambian infants suggested that during the mango (*Mangifera indica*) season there were improvements in gastrointestinal integrity and growth, and the acute-phase reponse normalized for a period of approximately 3 months (Northrop-Clewes *et al*. 1997). Mangoes are a rich source of β-carotene and the main supply of vitamin A for the year in this community. During infection, an acute-phase reaction is initiated which results in reduced levels of plasma retinol. Thus, increasing the availability of vitamin A throughout the year may help infants to restore gastrointestinal integrity, fight off infection and reduce the problem of faltering growth. It is hypothesized that vitamin A acts by restoring the physical and biochemical integrity of epithelial surfaces, particularly the gastrointestinal tract, so that the risk of infection and trauma will be reduced.

Effect of vitamin A supplementation on gastrointestinal integrity. Two vitamin A supplementation studies were carried out in Rourkela, Orissa State, India. Low serum retinol levels and growth faltering had previously been reported in this region (Das et al. 1996). The first study was carried out in four rural villages, where eighty infants aged 4–18 months were recruited (Northrop-Clewes et al. 1997). The infants were randomly allocated to receive either 5·0 mg retinol equivalents as retinyl palmitate and 40 mg α-tocopherol in 4 ml groundnut oil weekly for 8 weeks or 40 mg α-tocopherol in 4 ml groundnut oil as placebo. Baseline L : M values were 0·74 and 0·64 for the treatment and placebo group respectively. L : M values were reduced after 4 and 8 weeks in both groups, but only the change in the treatment group was significant ($P < 0·05$). Mean haemoglobin concentration also showed slight but non-significant improvement. However, the placebo group displayed a significant ($P < 0·05$) decrease in haemoglobin from 91·95 to 88·33 g/l after 8 weeks.

The second study investigated the effect of vitamin A supplementation on hospitalized infants with diarrhoea and respiratory infections (FSW McCullough, CA Northrop-Clewes, BD Das and DI Thurnham, unpublished results). Infants of weaning age (4–18 months; n 100) admitted with diarrhoeal disease or respiratory infection were randomly assigned to one of three treatment groups as follows: treatment 1, 60 mg retinol equivalents as retinyl palmitate (30 mg retinol equivalents for infants under 12 months) and 40 mg α-tocopherol on the day following admission; treatment 2, 60 mg retinol equivalents as retinyl palmitate and 40 mg α-tocopherol 5 d post-admission; treatment 3,

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placebo (no vitamin A, only 40 mg α-tocopherol 5 d post-admission). Gastrointestinal integrity and haemoglobin measurements were carried out at baseline, and 5, 10 and 30 d post-admission. Gastrointestinal integrity was measured using the dual-sugar intestinal-permeability test. Haemoglobin was measured using the Hemocue device (Hemocue, Angelholm, Sweden). Differences in L : M and haemoglobin between baseline and day 5 were not significant for any treatment. By day 10, however, both L : M values and haemoglobin levels were significantly ($P < 0{\cdot}05$) improved in the groups receiving the vitamin A, whereas there was no improvement in the placebo group. By day 30, the responses in the groups receiving vitamin A were still better than those of the placebo group, although not as significant ($P > 0{\cdot}05$).

Traditionally, micronutrient supplementation is prescribed on admission of patients to Ispat General Hospital (Rourkela City, Orissa State, India). The hospital policy should be re-examined and more vitamin A supplied, since the results of these studies suggest that gastrointestinal integrity is severely impaired during illness, but responsive to the additional vitamin A. Gastrointestinal integrity and haemoglobin were significantly ($P < 0{\cdot}05$) more rapidly improved in the vitamin A-treated groups than in the placebo group. However, there were no statistical differences between the two vitamin A groups, therefore there was no apparent advantage in giving vitamin A on admission. In addition, the majority of the children were discharged within 5 d.

In conclusion, marginal vitamin A status clearly impairs the integrity of epithelial tissues. These effects are more clearly seen in the eye as a result of the availability of the CIC test. Use of the dual-sugar intestinal-permeability test now opens up the possibility of examining more closely the influence of vitamin A on the gastrointestinal tract.

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