Effect of intramuscular vitamin A injection on plasma levels of vitamin A and retinol-binding protein in malnourished children

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1. Serum levels of retinol-binding protein (RBP), total vitamin A and retinyl ester were measured in twenty-four malnourished children with corneal lesions and nine normal children.

2. Initially, the RBP and vitamin A levels were significantly lower in malnourished children than in normal children.

3. After intramuscular injection of 30000 μg of aqueous vitamin A, serum levels of total vitamin A were increased significantly at 24 h and tended to fall within 5 d of treatment in both groups.

4. Before treatment, retinyl ester accounted for less than 10% of the vitamin in circulation. After vitamin A injection, it increased to 30%, a level much lower than that reported in patients with hypervitaminosis A.

5. There was no significant difference between the two groups of children and none of them showed clinical signs of toxicity. These observations indicate that administration of massive doses of vitamin A over a short period will not produce toxic effects even in malnourished children.

Excessive intake of vitamin A is known to produce toxic manifestations in animals and man (Wolbach & Bessey, 1942; Josephs, 1944; Rodahl, 1949; Muenter *et al.* 1971; Korner & Vollm, 1975). Hypervitaminosis A has been shown to be associated with increased concentration of total vitamin A in circulation, particularly retinyl ester which is not bound to the retinol-binding protein (RBP) (Mallia *et al.* 1975; Smith & Goodman, 1976). Massive doses of vitamin A are now being increasingly used in developing countries for both treatment and prevention of xerophthalmia in children (WHO, 1976; Reddy, 1978). However, concern has been expressed over the safe use of such large doses of the vitamin, since the capacity of the liver to hold the absorbed retinol to the protein is limited in protein–energy malnutrition (PEM). A study was therefore undertaken in malnourished children to determine the effect of massive doses of vitamin A on retinol transport, particularly the ester form of the vitamin in circulation.

SUBJECTS AND METHODS

Twenty-four children suffering from severe PEM (marasmic kwashiorkor) and who had conjunctival and corneal xerosis were investigated. Six children had ulceration of the cornea as well. Their ages ranged from 1 to 4 years. Sixteen of these children were treated with a single intramuscular injection of 30000 μg water-miscible vitamin A palmitate (Aquesol, Roche Products, Ltd), while the other eight received daily injections of the same dose for three consecutive days. In addition, they were given a diet which provided 4 g/kg protein and 840 kJ/kg for 3–4 weeks.

Nine normal children of the same age-group were also studied for comparison. They received a single injection of vitamin A. Blood samples were obtained from all the subjects initially and again on the second and fifth day after vitamin A injections were given. Free retinol and retinyl ester in serum were separated using deactivated alumina (Vahlquist, 1974) and vitamin A activity determined by a microfluorometric method (Selvaraj & Susheela, 1970). Serum RBP was measured by immunodiffusion technique (Mancini *et al.* 1965) and serum albumin by the dye method (Doumas *et al.* 1971).

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Table 1. *Serum retinol-binding protein (RBP) and vitamin A levels in normal and malnourished children*

(Mean values with their standard errors)

Group	No. of subjects	Albumin (g/l)	RBP (μg/ml)	Vitamin A (μg/l)
Normal children	9	32±0·8	33·4±2·26	287±18·8
Malnourished children	24	16±0·7	18·0±0·82*	88±16·3*

* $P < 0·001$ as compared to normal.

Table 2. *Effect of a single dose of vitamin A* on serum levels of the vitamin in normal and malnourished children*

(Mean values with their standard errors; no. of children in parentheses)

Group	Period after injection (d)	Total vitamin A (μg/l)		Retinyl ester			
				μg/l		% vitamin A	
		Mean	SE	Mean	SE	Mean	SE
Normal children (9)	0	277	17·6	32	12·8	9·6	3·84
	2	1526	180·2	408	42·9	34·4	3·48
	5	340	27·7	74	11·1	21·4	3·22
Malnourished children (16)	0	80	12·0	ND		ND	
	2	2154	360·9	642	178·8	30·2	3·53
	5	534	61·1	143	30·3	23·2	3·39

ND, not detectable.

* 30000 μg aqueous vitamin A injection.

Statistical analyses were carried out using Student's *t* test for comparison of the values of normal and malnourished children, and paired *t* test for testing the effect of vitamin A injections.

An informed consent was obtained from parents of all the children investigated. Administration of massive doses of vitamin A to normal children is an accepted prophylactic measure followed in India.

RESULTS

All children with eye signs showed clinical improvement following vitamin A administration. In those who had xerosis, the lesions cleared up completely and vision was restored to normal within a few days. But those who had corneal ulceration developed opacities and retained only partial vision. There was no difference in the clinical response to a single dose or three doses of vitamin therapy. None of them developed clinical signs of toxicity.

Initially serum albumin, vitamin A and RBP levels were significantly lower in malnourished children than in normal subjects (Table 1). After a single injection of vitamin A, serum vitamin A levels increased significantly on the second day and tended to fall thereafter. The increase was slightly higher in malnourished than in normal children, but the difference was statistically not significant (Table 2). In children who received three doses of the vitamin the response was similar, and serum levels of vitamin A returned to normal within 5 d after the last dose (Table 3).

Table 3. *Effect of three doses of vitamin A* on serum levels of the vitamin in malnourished children*

(Mean values with their standard errors for eight children)

Period after 1st injection	Total vitamin A (μg/l)		Retinyl ester			
			μg/l		% vitamin A	
	Mean	SE	Mean	SE	Mean	SE
0	96	20·7	ND		ND	
2	1937	239·5	765	79·0	39·3	4·12
5	1637	379·9	368	101·4	23·0	3·35
8	475	49·1	147	26·7	29·5	3·55

ND, not detectable.

* Daily injections of 30000 μg aqueous vitamin A for first three days.

Before treatment, vitamin A was present in circulation mostly in the form of alcohol. The ester fraction accounted for approximately 10% of the vitamin in normal subjects while in malnourished children the levels were undetectable. After the administration of vitamin A, retinyl ester accounted for 20–30% of the vitamin (Table 2). There was no significant difference in this regard between the two groups of children. Those who received three doses of vitamin A showed a similar trend (Table 3).

DISCUSSION

Excessive doses of vitamin A taken orally or parenterally can result in acute toxicity. The dose that is required to produce toxicity is not known with certainty since there are wide individual variations. In some children, as little as 100000 μg is sufficient (Marie & See, 1954; Swaminathan *et al.* 1970), whereas in others considerably larger amounts are needed (Furman, 1973). Factors influencing vitamin A toxicity include age, state of nutrition, type of vitamin A preparation and route of administration. Present-day concern over safe use of vitamin A stems from the current practice of administration of massive doses of vitamin A as an emergency or short-range approach to the eradication of nutritional blindness in children.

Acute vitamin A toxicity is characterized by anorexia, nausea, vomiting and irritability. Clinical diagnosis of toxicity is, however, difficult in an acutely ill malnourished child as he may have these symptoms even before vitamin A is administered. Biochemical detection of toxicity is based on circulatory levels of vitamin A, particularly the ester fraction. In an adult consuming 1 million units of vitamin A daily for a period of 25 d, the serum level of the vitamin was reported to be as high as 13 mg/l (Hillman, 1956). Apparently, hypervitaminosis A occurs in vivo only when the intake of the vitamin overloads the liver's capacity to store excess in the form of retinyl esters. In this context, the relative level of retinol *vs.* retinyl ester assumes significance. Smith & Goodman (1976) studied vitamin A transport in three subjects with hypervitaminosis A. In each of these patients the clinical toxicity was associated with increased concentration of total vitamin A, and particularly of retinyl esters not bound to RBP.

In the present study, none of the children showed clinical signs of toxicity following vitamin A administration. Initial levels of serum vitamin A and RBP were significantly lower in malnourished children, an observation similar to that reported earlier (Smith *et al.* 1973; Reddy *et al.* 1979). Following the administration of vitamin A, the total vitamin as well

as the retinyl ester fraction showed a significant increase in 24 h and tended to fall within 5 d; this trend was observed in both normal and malnourished children. Even in those who received three doses of vitamin A, the retinyl ester fraction did not exceed 40%, which is far below the level (65–67%) reported by Smith & Goodman (1976) in patients with vitamin A toxicity. These findings indicate that administration of massive doses of vitamin A over a short period will not produce toxic effects even in malnourished children.

The best way to prevent vitamin A deficiency would be to provide small quantities of the vitamin daily in the diet. However, there are situations in developing countries where the diets do not meet vitamin A requirements and, therefore, resort has been made to intermittent massive dose intervention (WHO, 1976; Reddy *et al.* 1978). Malnourished children with corneal lesions are treated immediately on diagnosis with two or three massive doses of vitamin A (WHO, 1976; Reddy, 1978). Observations made here suggest that there is no risk of hypervitaminosis A with this treatment regimen. However, repeated doses of the vitamin are unnecessary since a single injection of 30000 μg of aqueous vitamin A is effective in the treatment of severe xerophthalmia.

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