Vitamin A absorption in children with ascariasis

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The effect of *Ascaris lumbricoides* infection on retinol absorption was investigated in young children from a slum area of Dhaka City, Bangladesh. Twenty-four children aged 4–10 years were screened and in every case eggs of either *Ascaris*, *Trichuris* or hookworm were isolated from the stool. The average serum retinol was 0·91 (SD 0·35) μmol/l and sixteen children had levels below 1·05 μmol/l. This compared with a serum retinol concentration of 1·70 (SD 0·52) μmol/l in five reference children from a more privileged social background. An oral dose of retinol (41·8 μmol) was given to ten children in whom the concentration of *Ascaris* eggs in the stool varied. Less than 1% of the supplement could be recovered in the stools collected over the following 48 h. *Ascaris* worms were isolated from the stool and assayed for retinol content. In no case was retinol detected in the worms. These findings do not support the contention that infection with *Ascaris* predisposes to malabsorption of vitamin A.

Vitamin A: Childhood: Ascariasis

Vitamin A deficiency is thought to be one of the most common nutritional deficiencies in the world (Roels, 1970). There is evidence to suggest that the deficiency is associated with increased susceptibility to surface infections of the respiratory and gastrointestinal tract and that supplements of vitamin A may decrease the mortality and morbidity from a range of common infections (West *et al.* 1989; Ahmed *et al.* 1990*b*). It is undoubted that vitamin A deficiency is a major cause of preventable blindness in childhood (Pirie, 1983). There is an ongoing debate about the need for giving vitamin A supplements as a public health approach in the prevention of blindness (Gopalan, 1990) and potential benefit in reducing the risk of mortality and morbidity from respiratory and diarrhoeal diseases (Sommer, 1989; Rahmathullah *et al.* 1991). Therefore, it is of considerable importance to be able to identify the factors which contribute to an overall deficiency state in children at risk.

It has generally been considered that a deficiency of vitamin A is caused mainly by a primary dietary deficiency of the nutrient (Gopalan *et al.* 1960). However, it is also recognized that the vitamin A status of individuals may be made worse by infections (Arroyave & Calcano, 1978; Campos *et al.* 1987) and there is evidence which suggests that children who have infections with gastrointestinal helminths, in particular *Ascaris lumbricoides*, are at particular risk of vitamin A deficiency. It has been said that the main reason why *Ascaris* predisposes to vitamin A deficiency is because it causes malabsorption of vitamin A (Mahalanabis *et al.* 1976, 1979).

There are a number of technical problems which have inhibited the development of a clear understanding in this area. First, it is difficult to obtain a reliable index of vitamin A status (Underwood, 1990). Functional tests are not necessarily specific and serum levels of

vitamin A cannot always be interpreted with reliability. Vitamin A is carried in the plasma bound to retinol-binding protein (RBP), a negative acute-phase reactant whose formation is sensitive to the dietary intake of protein and Zn (Jacob *et al.* 1978). Furthermore, vitamin E is thought to exert an influence on the retention of vitamin A (Sivakumar & Reddy, 1978). Second, the absorption of vitamin A has seldom been measured directly; rather the standard approach has been to give an oral dose of vitamin A and to follow the change in plasma concentration in peripheral blood (Mahalanabis *et al.* 1976). By analogy with the effect of variations in dietary complex carbohydrate on a glucose-tolerance curve, it is clear that there is no necessary reason why the dose–response curve should relate directly to the overall absorption of the vitamin, although it may give some indication of the rate of absorption or some aspect of hepatic handling of the administered dose. The same test has been used as the relative dose–response assay to determine overall vitamin A status (Campos *et al.* 1987; Underwood, 1990).

Sivakumar & Reddy (1972) have measured the faecal loss and retention of isotope from an oral dose of labelled vitamin A. In comparison with healthy children who absorbed 99% of the radiolabel, children with pneumonia and those with *Ascaris* absorbed 74 and 80% of the radiolabel respectively (Sivakumar & Reddy, 1975). For the children with *Ascaris* there was no correlation between the expelled worm load and the apparent absorption of vitamin A.

Using the relative dose–response, Mahalanabis *et al.* (1976) have demonstrated an impairment of vitamin A absorption in adults with *Ascaris*. With a water-miscible preparation of vitamin A, absorption was found to be impaired in children infected with either *Ascaris* or *Giardia* (Mahalanabis *et al.* 1979). These infections have been said to lower serum retinol levels (Arroyave & Calcano, 1978) and to deplete liver stores in children (Campos *et al.* 1987). However, as Sivakumar & Reddy (1972) did not find an increase in urinary loss of labelled vitamin A in children with pneumonia, it may be that increased endogenous losses take place through the gastrointestinal tract rather than in the urine.

There are reports that *Ascaris* itself contains vitamin A (Leutskaya, 1961), leading to the suggestion that some intestinal parasites may accumulate vitamin A from the host's tissues (Comley & Jaffe, 1983). However, Sivakumar & Reddy (1978) were only able to isolate small traces of label from administered vitamin A in the expelled worms, and the content of vitamin A determined spectrophotometrically appeared to be about 1·16 nmol/worm.

In common with other developing countries, Bangladesh has a high prevalence of vitamin A deficiency in both the urban and the rural populations. The toll taken by vitamin A deficiency in preventable blindness and ill health has made it one of the major public health problems of childhood. To our knowledge there are few reports in which the specific retinol content of stools has been measured directly in children with ascariasis. We have recently developed a method to measure retinol in the stools using HPLC (Ahmed *et al.* 1990*a*). Therefore, the present study was designed to determine whether children with ascariasis excrete increased amounts of retinol in the stools, and to assess the extent to which retinol accumulated by the worms might account for this.

METHODS

Subjects

The study involved three protocols.

Protocol 1. Twenty-four children aged 4–10 years from a poor urban slum area of Dhaka City, Bangladesh, were surveyed to identify whether they were infected with worms and to characterize their vitamin A status. The area selected had very poor hygiene and sanitary facilities. The twenty-four children were compared with five children of a similar age from

a relatively high socio-economic background (controls). On a predetermined day the children were examined. Age, height and weight were recorded, 1 ml blood was taken by venepuncture for vitamin A assay and a stool sample was collected.

Protocol 2. Ten of the twenty-four children participated in a vitamin A absorption test. The children were selected to represent a range of infective load as assessed by the parasite egg density in the stools. The test was performed by giving each child a single oral dose of 12·0 mg vitamin A (retinol equivalent) as retinol palmitate in oil. (Arovit; Roche). Following supplementation each subject was monitored every 6 h and a complete collection of all stools passed was maintained for 48 h. The stool samples were taken to the laboratory within 2 h and the stools from each individual were pooled and weighed, then homogenized in deionized water to a smooth consistency before being stored at −20°.

Protocol 3. Male and female ascarids were isolated from the stools of ten children, aged between 2 and 12 years, resident in a slum area of Dhaka City. The worms were prepared for analysis of retinol content (see pp. 819–820).

Laboratory analyses

The egg count for intestinal parasites was carried out using the formol–diethyl ether technique (Hall, 1981), with the quantitative identification of eggs from *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm. The results were expressed as eggs/g stools. The egg density was taken as a crude estimate of the infective burden for the child.

Serum retinol was determined using HPLC according to the method of Bieri *et al.* (1979) with slight modifications. In brief, retinol was extracted with hexane, and retinyl acetate (RA; 500 μg/l) was added as an internal standard. Retinol was separated by HPLC using a reverse-phase C_{18} column with a solvent system of methanol–water (95:5, v/v) and analysed spectrophotometrically with a Pye Unicam solvent delivery system, detector and recorder. The coefficient of variation for retinol determined from the assay of eight replicates from a pooled serum was 4·1%.

The retinol content of the stools was measured by HPLC as described by Ahmed *et al.* (1990*a*). Specimens of stools were thawed and mixed thoroughly by hand. A portion of the stools was saponified with 1 M-KOH in ethanol–distilled water (90:10 v/v) containing pyrogallol (10 g/l), at 60° for 30 min. Retinol was extracted twice with hexane. The hexane extract was pooled and dried in a rotary evaporator at 40° before being redissolved in methanol and the retinol assayed by HPLC. It has been found that RA cannot be used as an internal standard when added before saponification (Ahmed *et al.* 1990*a*), therefore duplicate samples of all specimens were run with and without added retinol. For ten samples the mean recovery of retinol was 87·4% with the overall coefficient of variation being 4·4%. The precision of the HPLC instrument was determined by assaying twelve stool samples, where RA was added to the dried extracted retinol following saponification. Under these conditions the recovery of RA was 96% with the overall coefficient of variation for recovery being 4%.

The retinol content of the vitamin A supplement given to the children was assayed by dissolving eight drops of the solution in 100 ml absolute ethanol. A portion was heated with alcoholic KOH and extracted with hexane as described previously for the assay of retinol in the stools. The recovery of added retinol was determined in an additional portion.

The retinol content of male and female ascarids was determined by homogenizing the worms using a pestle and mortar. The homogenate was heated in alcoholic KOH containing pyrogallol (10 g/l) in a water-bath at 60° for 30 min. After cooling to room temperature, distilled water (5 ml) was added, followed by 10 ml hexane. The sample was mixed thoroughly for 1 min and then centrifuged at 1000 ***g*** for 10 min. The top hexane layer was collected and the extraction procedure repeated with a further 10 ml hexane. The

Table. 1. *The weights and heights, serum retinol values and stool levels of eggs of the gastrointestinal parasites* Ascaris, *hookworm and* Trichuris *in young Bangladeshi children aged from 4 to 10 years**

Subject no.	Age (years)	Sex	Wt kg	Wt (%)†	Height m	Height (%)†	Serum retinol (μmol/l)	Worm eggs in stools (eggs/g stools) *Ascaris*	Hookworm	*Trichuris*
Group from slum area										
1	4	M	12·3	76	0·90	89	0·36	1481	0	329
2	5	M	14·4	79	0·96	88	0·47	3521	195	489
3	6	M	12·3	61	1·02	88	0·74	51	0	77
4	6	M	16·2	81	1·08	94	1·23	9119	97	582
5	6	M	16·2	81	1·08	94	0·72	0	0	417
6	6	M	13·5	67	1·00	87	0·42	415	0	1487
7	6	M	19·8	99	1·16	101	0·58	711	0	177
8	7	F	11·6	58	0·97	84	0·82	1730	0	192
9	7	F	14·1	63	1·04	86	0·85	301	15	0
10	7	F	15·5	70	1·07	88	1·20	77	0	467
11	7	F	15·4	69	1·01	83	0·95	1344	0	566
12	7	F	15·4	69	1·10	91	0·60	1610	0	196
13	7	M	15·8	71	1·10	91	1·11	8353	47	1397
14	7	F	16·5	74	1·06	88	0·91	1188	58	294
15	7	M	16·6	74	1·06	88	0·57	700	0	400
16	7	M	16·9	76	1·15	95	1·35	550	0	338
17	7	M	17·5	79	1·12	93	0·90	2420	0	302
18	7	M	18·7	84	1·15	95	1·19	125	0	75
19	8	M	19·0	76	1·16	92	0·74	216	28	187
20	8	F	19·0	76	1·18	93	1·00	896	0	579
21	8	M	20·8	83	1·21	96	0·96	709	70	496
22	8	M	22·0	88	1·21	96	1·05	1545	0	960
23	9	M	18·5	65	1·20	95	1·69	2474	154	309
24	9	F	23·2	82	1·27	100	1·38	3401	0	850
Reference group										
1	4	F	12·7	78	0·99	97	1·35	60	0	0
2	5	F	15·4	85	1·09	100	2·61	0	0	0
3	6	M					1·40	0	0	0
4	9	F	18·3	65	1·23	93	1·47	96	0	144
5	10	M					1·69	0	0	0

* For details of methods, see pp. 819–820.

† Weight-for-age and height-for-age are expressed as a percentage of the National Centre for Health Statistics standard.

hexane extracts were pooled and evaporated to dryness in a rotary evaporator at 40°. The retinol in the dried extract was redissolved in methanol and the retinol content estimated by HPLC.

RESULTS

Table 1 show the characteristics of the twenty-four children from the slum area and the five controls examined in the initial survey. The average weight-for-age of the poor children was 75 (SD 10)% of the National Centre for Health Statistics reference (Hamill *et al.* 1979). Among the five children from a higher socio-economic background one child had eggs of *Ascaris* (60 eggs/g stools) and another child had eggs of *Ascaris* and *Trichuris* (90 and 144 eggs/g stools respectively). Among the twenty-four children from the slum area the excretion of *Ascaris* eggs ranged from 0 in one child to 9119 (mean 1789, median 1042)

Table. 2. *The effect of administering a single oral dose of retinol (41·8 μmol) to young Bangladeshi children infected with* Ascaris *on the excretion of retinol in stools over the 48 h period after the dose**

Sample	Stool retinol (nmol)	Recovery (%)	Corrected stool retinol (nmol)	Apparent absorption (%)	Worm eggs in stools (eggs/g)	
					Ascaris	*Trichuris*
1	54	95	57	99·86	1481	329
3	29	85	34	99·92	51	77
5	278	93	299	99·28	0	417
7	122	85	144	99·65	711	177
8	68	91	75	99·82	1730	192
9	38	88	43	99·90	301	0
13	317	84	377	99·09	8353	1397
18	65	95	68	99·83	125	75
20	133	84	158	99·62	896	579
24	307	90	341	99·18	3401	850

* For details of methods, see pp. 819–820.

eggs/g stools. In these children *Trichuris* eggs ranged from 0 to 1487 (mean of 465, median 369) eggs/g stools. In eight of the twenty-four children eggs of hookworm were identified, up to 195 eggs/g stools. The five control children all had serum retinol concentrations above 1·05 (mean 1·70, range 1·35–2·61) μmol/l. In contrast, 80% of the children from the slum area had serum retinol values below 1·05 (mean 0·91 (range 0·36–1·69)) μmol/l. There was no correlation between the serum retinol concentration and the concentration of *Ascaris* eggs found in the stools; expressing the egg concentration in stools as $\log_{10}$ did not improve the relationship. Similarly, there was no relationship between the number of hookworm eggs or the number of *Trichuris* eggs and serum retinol concentration. There was a significant linear relationship between the concentration of *Ascaris* eggs and the concentration of hookworm eggs ($P = 0{\cdot}028$), and between *Ascaris* eggs and *Trichuris* eggs ($P = 0{\cdot}032$).

Ten children were given eight drops of vitamin A solution (Arovit; Roche, estimated to contain 12·0 mg) orally. By analysis, eight drops of this solution was found to contain 41·8 (SD 0·36) μmol retinol (n 8). Table 2 shows that the recovery of retinol in the stools passed during the 48 h after the supplement was a median of 0·11 (range 0·035–0·38) μmol. This represented less than 1% of the dose in all the children, giving an apparent absorption (%) for retinol (intake − stool losses) in excess of 99 (median 99·72, range 99·1–99·9). For the ten children there was a negative linear relationship between eggs/g stools and apparent absorption of retinol (r −0·68, $P = 0{\cdot}030$), but even in the children with the highest concentrations of *Ascaris* eggs there was very little retinol recovered in the stools. A similar negative relationship could be demonstrated for the apparent absorption of retinol and the number of *Trichuris* eggs in the stools (r −0·87, $P = 0{\cdot}001$), related to the fact that the concentrations of *Ascaris* and *Trichuris* eggs were closely associated (r 0·91, $P = 0{\cdot}0003$).

It was not possible to detect any retinol in any of the male or female *Ascaris* worms recovered from stools following therapeutic expulsion. In the first series of assays 0·9–3·5 g worms were homogenized. The method was modified and similar weights of worms were homogenized in anhydrous Na_2SO_4 and no retinol could be detected. Finally, a known amount of retinol was added, before homogenization, to the worm samples in order to estimate the recovery of retinol. Ethanol (15–20 ml) was added to each of the homogenized

worm samples which were left overnight at 4°. Retinol was then extracted twice with hexane and the hexane extracts pooled and evaporated to dryness with a rotary evaporator at 40°. The extract was redissolved in methanol and retinol assayed by HPLC. The mean recovery of retinol from the samples was 104 (96 and 112)%. The extraction for retinol was repeated with up to twenty worms weighing 19·4 g in a single batch, and again no retinol could be detected. The extraction was repeated with 16·5 g worms being homogenized and extracted with chloroform rather than hexane. Vitamin A was extracted with chloroform solution, and trichloroacetic acid (TCA) in chloroform was used as a colorimetric reagent (Bayfield, 1971). The TCA in chloroform reacts with vitamin A solution to give a blue colour which can be measured spectrophotometrically at 620 nm, using TCA in chloroform as the blank. It was not possible to identify retinol in the worms either by HPLC or by colorimetry.

DISCUSSION

It has been stated that infection with *Ascaris* gives rise to vitamin A malabsorption, and this has been considered to be one of the important factors which contributes to vitamin A deficiency as a public health problem in locations where sanitary facilities and simple hygiene are poor (Feachem, 1984, 1987). We are not aware of any study in which the malabsorption has been measured directly by the analysis of faecal losses of vitamin A. Sivakumar & Reddy (1975) measured the recovery of isotope from stools and Mahalanabis *et al.* (1979) have used the relative dose–response test to represent absorption. In the present study retinol in the stools was measured following an oral challenge with vitamin A. In all the children studied the apparent absorption of vitamin A was in excess of 99%. Of the twenty-four children, twenty-three had evidence of infection with *Ascaris* based on the excretion of eggs in the stools. We measured vitamin A in the stools of children with cystic fibrosis who absorb only 50% of their dietary vitamin A intake and, thus, when malabsorption exists it can be detected (Ahmed *et al.* 1990*a*). Therefore, it is not possible to sustain the possibility that infection with *Ascaris* predisposes to vitamin A deficiency through the promotion of vitamin A malabsorption.

The presence of *Ascaris* eggs in the stools does not in itself relate directly to the worm burden, but it has been used to give some indication of the severity of the worm load carried by the host. In none of the cases in the present study was the egg density in stools particularly great and it may be argued that the infections were not sufficiently heavy to promote malabsorption. The subjects studied by Mahalanabis and colleagues had egg counts in the range 100–16000 in adults (Mahalanabis *et al.* 1976) and up to 75000 in children (Mahalanabis *et al.* 1979). We have no way of knowing whether malabsorption of vitamin A only occurs with very heavy worm burdens and this is an important point to resolve in future studies. The other suggestion would be that retinol which is not absorbed might be metabolized by the microflora of the lower bowel and this would be one way to explain why Sivakumar & Reddy (1975) were able to recover radiolabel from the stools. We have no way of knowing whether this theoretical possibility occurred in fact. We were not able to identify significant amounts of retinol in the stools and we are inclined to consider it an unlikely possibility. The metabolic end-products of retinol metabolism are not clear, and it may be that biliary excretion is one important route through which lipid- and water-soluble breakdown products are lost to the body. If so, this might explain the finding of increased amounts of label in the faeces, associated with no increase in that in urine, in the studies of Sivakumar & Reddy (1972), especially as these authors note that a significant part of the label was recovered from non-saponifiable fractions of urine and stools.

In nutrition, the classic approach to determining the net absorption of a nutrient presumes that material appearing in the stools represents unabsorbed dietary residue and

that there is no influence on stool composition resulting from the metabolic activity of the gastrointestinal tract and its resident microflora. Increasingly the evidence demonstrates that this assumption is not justified. Stools have a complex composition which results from the metabolic interaction of the colonic microflora with unabsorbed dietary residue and endogenous material derived from sloughed cells, mucus and gastrointestinal secretions (Jackson *et al.* 1992). Based upon our knowledge of the metabolism of vitamin K, the microflora appear capable of synthesizing fat-soluble vitamins which are available to the host. It is less clear whether the microflora are capable of draining micronutrients, such as retinol, from the host. Elucidation of this point would require an assessment of the balance of retinol across the colon, perhaps through the use of ileostomy models. As bacteria account for 30% or more of the stool mass they clearly do not contain significant quantities of retinol; it remains an open question at this time whether they utilize or degrade retinol.

Sivakumar & Reddy (1978) were able to measure small amounts of retinol in *Ascaris* worms using a colorimetric method, and Leutskaya (1961) also reported that *Ascaris suum* contained retinol. Sivakumar & Reddy (1978) found that the stool losses of retinol were not related to the worm burden and the amounts of retinol found in each worm were insufficient to account for the total loss of label in the stools. Using HPLC, a more specific method, we were not able to detect traces of retinol in the worms. Male and female worms were examined separately, and different approaches to homogenization and extraction failed to demonstrate the presence of retinol. Ascarids do not have a liver or kidney as potential storage organs for retinol and so the whole body was used for extraction (Leutskaya, 1961). It was considered that under these circumstances time might be required for the adequate extraction of retinol from the worms, but keeping the preparation at 4° in ethanol overnight to facilitate the recovery of retinol from the worm did not lead to a positive result. Increasing the mass of worms from which the extraction was carried out gave negative results. A recovery experiment was carried out to demonstrate that if retinol had been present the procedures used should have ensured adequate extraction in measurable quantities. Therefore, it can be concluded that it is unlikely that the *Ascaris* worms concentrate retinol from the host and, therefore, this route of loss is not likely to make a significant contribution to vitamin A deficiency. The close relationship between the egg count in the stools and stool retinol might be a spurious association. It is unlikely to be simply the result of malabsorbed retinol, but might represent host losses as a result of mild blood loss into the colon or rectum induced by *Trichuris*.

It was not possible to carry out functional tests of vitamin A deficiency in the children in the present study. It has been assumed that the majority of the poor children were vitamin A deficient on the basis of a low serum concentration. A low serum retinol is not specific for vitamin A deficiency and certainly it is not possible to rule out the possibility that the children were infected and the retinol in serum was lowered as a part of a more general acute-phase reaction. The extent to which infection with *Trichuris* and/or *Ascaris* might evoke a general inflammatory response in their own right is not clear. However, the two children in the control group who had small numbers of eggs in the stools both had a serum retinol in the normal range. A number of investigators imply that infection increases the turnover of retinol in the body, thereby increasing losses from the body and contributing directly to a deficiency state. To our knowledge there is no reliable information for humans which supports this possibility other than the implications carried in the findings of Sivakumar & Reddy (1972). Bundy & Golden (1987) have raised the question of the extent to which children who are undernourished are at a particular risk of infection with intestinal parasites. They have shown that when severely undernourished children with *Ascaris* were treated with food and general support they were able to clear infection with *Ascaris* spontaneously without recourse to antihelminthic chemotherapy. Virtually all the

children in the present study demonstrated a mild-to-moderate degree of undernutrition which might have placed them at increased risk of worm infection. A 24 h dietary record was taken from all the children. Their diets appeared to be monotonous and lacked both retinoids and carotenoids.

We have been unable to demonstrate malabsorption of a dietary supplement of retinol in poor urban children in Bangladesh and we have no reason to believe that gastrointestinal infection with *Ascaris* predisposes to a deficiency of vitamin A through the promotion of malabsorption. There is the need to develop much more direct methods for measuring vitamin A turnover in health and infection in order to be able to define with greater specificity the changes which take place during infections. Information of this kind would be of the greatest value in helping to develop rational public health strategies directed towards the prevention of vitamin A deficiency.

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