Absorption of labelled vitamin A in children during infection

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1. The intestinal absorption of [11,12-$^3H_2$]retinyl acetate was studied in five apparently normal children, eight children with respiratory infection and three with gastroenteritis.

2. The absorption of vitamin A was significantly lower in children with respiratory infection or gastroenteritis than in normal children.

3. In the light of these observations, it is suggested that repeated attacks of infections may significantly contribute to the prevalence of vitamin A deficiency in children of poor communities.

Vitamin A deficiency is one of the most important paediatric nutritional problems in India; the results of several surveys have shown that the dietary intake of vitamin A is inadequate in children of low-income groups (Gopalan, Venkatachalam & Bhavani, 1960).

It is generally believed that malnutrition predisposes to infection. Episodes of gastro-intestinal and respiratory infection are frequently seen among children of the poorer sections of the population whose nutritional status is far from optimal. It has been reported that the concentrations of vitamin A in blood (Popper, Steigmann, Dubin, Dyniewicz & Hesser, 1948) and liver (Moore, 1937) are lowered during episodes of respiratory infection. The occurrence of repeated infections may thus adversely affect vitamin stores which are already low and may have an important contributory part to play in the prevalence of vitamin A deficiency among children of poor communities.

An investigation was undertaken to study the effects of infection on the metabolism of vitamin A, with particular reference to the pattern of intestinal absorption and urinary excretion of vitamin A in children.

EXPERIMENTAL

Subjects

Five apparently normal children and eleven children with infection were investigated. Seven of the eleven children were suffering from respiratory infection (subjects 1–7), one child had enteric fever and bronchitis (subject 8) and the other three had gastroenteritis (subjects 9–11). Their ages ranged from 2 to 10 years. Each child was given orally 4–5 μCi [11,12-$^3H_2$]retinyl acetate (specific activity, 380 μCi/mg) in oil, along with 1000 μg non-radioactive retinyl acetate. Labelled vitamin A was obtained from Hoffman–La Roche & Co. Ltd and purified by column chromatography (Olson, 1961) before administration. Twenty-four-hour samples of urine and faeces were collected for 4–6 d after the dosing.

All the subjects with infection received antibiotics (400000 U penicillin and 0·5 g streptomycin daily) during the course of the study. To determine whether this had influenced the metabolism of vitamin A in any way, three other normal children were given intramuscular injections of streptomycin and penicillin daily for 4–5 d and the studies were repeated.

Measurement of radioactivity in urine and faeces

Radioactivity in samples of faeces and urine was measured with a Packard Tri-Carb liquid scintillation spectrometer with a counting efficiency of 33% for tritium. Quench corrections were made with appropriate internal standards.

The total radioactivity in the urine samples and in the ethanolic extracts of faeces was determined using a dioxan-based scintillation mixture (Bray, 1960).

Urine and faecal samples were saponified and extracted with hexane. The hexane extract was taken into toluene scintillator (Birks, 1964) and the unsaponifiable count obtained. The difference between the total count and the unsaponifiable count was taken as the water-soluble fraction count. The hexane extracts of the faecal samples were also used to measure the vitamin A content by a spectrophotometric method (Cama, Collins & Morton, 1952).

Faecal fat was estimated by a gravimetric method (Rencher & Beeler, 1969).

Blood samples were obtained before the administration of the dose and serum vitamin A was determined by a fluorometric method (Selvaraj & Susheela, 1970).

RESULTS

Studies in children with respiratory infection

Intestinal absorption of labelled vitamin A. Very little label was found in the faeces of normal children, indicating that the intestinal absorption of retinyl acetate was almost complete. In eight children with infection (subjects 1–8) considerable amounts of label were recovered in the faeces up to the 4th day. The mean level of absorption was only 74·3% in this group compared to 99·2% in the control group (Table 1). Values for absorption in individual children with infection (Table 2) showed that in none of the children was absorption complete, 10–70% of the administered label

Table 1. *Absorption of labelled vitamin A in children (mean values with their standard errors)*

(4–5 μCi [11,12-^{3}H]retinyl acetate, approximately 11μg, were administered orally to each child with 1000 μg unlabelled retinyl acetate)

Group	No. in each group	Serum vitamin A as retinol (μg/100 ml)	% of dose absorbed from the gut	% of absorbed label excreted in urine	% of dose retained in the body
Normal children	5	21·6 ± 4·83	99·2 ± 0·47	17·0 ± 1·58	82·2 ± 1·99
Children with infection	8	20·7 ± 3·33	74·3 ± 6·83*	23·3 ± 2·96	57·6 ± 5·96**

* $P < 0·05$. ** $P < 0·01$.

appearing in the faeces. The duration and type of infection did not seem to have influenced the extent of failure of absorption.

None of these eight children who showed impaired absorption had abnormal bowel movements. The fat content of the stools was less than 5 g/24 h.

Urinary excretion of the label. Normal children excreted in their urine 17% of the label absorbed from the gut, on average, whereas in those with infection the mean excretion was 23·3%. The difference between these values was not statistically significant. However, the excretion of the label was less than 20% in all the five normal children, whereas in six of eight subjects with infection, urinary excretion was more than 20% of the dose absorbed. Most of the label was excreted within 48 h after dosing; no radioactivity could be detected in the urine after 4 d.

Table 2. *Absorption of labelled vitamin A and excretion of tritium in faeces of eight children with infection*

(Dose as in Table 1)

			Vitamin A		Tritium	
Subject no.	Nature of infection	Duration of illness (d)	% of dose absorbed from the gut	% of absorbed label excreted in urine	Total count (% of dose administered)	Unsaponifiable count as % of total count in faeces
1	Lung abscess*	10	90·1	24·0	9·9	24·0
2	Bronchopneumonia	7	85·5	24·0	14·5	19·5
3	Bronchopneumonia	4	80·0	21·0	20·0	11·1
4	Bronchopneumonia	7	72·2	10·6	27·8	78·0
5	Bronchopneumonia	3	72·0	39·0	28·0	33·0
6	Bronchopneumonia*	3	30·7	24·8	69·3	76·2
7	Bronchopneumonia*	7	91·3	15·6	8·7	30·6
8	Enteric fever and bronchitis*	30	72·7	27·1	27·3	65·3
Mean			74·3	23·3	25·7	42·2

* Body temperature was elevated at the time of investigation.

Radioactivity in the unsaponifiable fractions of urine and faeces. No label could be detected in the unsaponifiable fraction of urine. All the radioactivity was found in the water-soluble phase, both in normal children and in children with infection. In the faeces, the radioactivity in the unsaponifiable fraction varied from 11 to 78% of the total count (Table 2). The vitamin A content of the faecal samples determined spectrophotometrically corresponded to the amount calculated from the radioactivity present in the unsaponifiable fraction of the sample, indicating that vitamin A was excreted as such, to some extent; the rest of the excreted label was attributable to degraded products of vitamin A.

The amount of vitamin retained was calculated as the difference between the amount of labelled vitamin given and the amount of the label excreted in the urine and faeces. The percentage of retention was found to be significantly higher in normal children than in those with infection (Table 1).

Serum levels of vitamin A in children with infection were not significantly different before dosing from those of controls.

Normal children who had received antibiotics did not exhibit abnormal excretion of the label either in faeces or in urine.

Studies in children with gastroenteritis

Three subjects (9–11) who were investigated while suffering from acute diarrhoea showed impaired intestinal absorption of vitamin A. Two children who had seven to eight loose stools/d showed 64·4% and 64·8% absorption, and the other subject, who had four to five stools/d, had an absorption of 81·4% of the label administered. The percentage of the absorbed label excreted in the urine was similar to that seen in normal children (Table 3).

Table 3. *Absorption of labelled vitamin A in children suffering from diarrhoea*

(Dose as in Table 1)

Subject no.	% of dose absorbed from the gut	% of absorbed label excreted in urine
9	64·4	20·0
10	81·4	18·5
11	64·8	9·6

DISCUSSION

It had been reported earlier that blood concentrations (Popper *et al.* 1948) and liver stores (Moore, 1937) of vitamin A were diminished in subjects suffering from respiratory infections. It was suggested that these changes occurred as a result of increased catabolism and excessive loss of vitamin A in urine (Lawrie, Moore & Rajagopal, 1941). However, no studies have been reported previously to indicate that the metabolism of vitamin A is altered during infection.

The results of our study show that a higher percentage of absorbed label was excreted in the urine of children suffering from respiratory infection than in that of normal children, but the absolute amounts of label excreted were similar in both the groups. In the absence of information on the pool size, it is difficult to interpret these results in terms of vitamin A catabolism.

Lawrie *et al.* (1941) reported that intact vitamin A is excreted in urine of subjects with pneumonia. Our results do not confirm this. The radioactivity was found exclusively in the water-soluble fraction, which indicates that vitamin A was not excreted as such, but as metabolites. Studies are now in progress to identify the metabolites.

The more striking observation made here is that during infection there was gross impairment in the absorption of vitamin A. This defective absorption was not due to

malabsorption of fat, since steatorrhoea was not present in any of the subjects. The mechanism by which the absorption of vitamin A is depressed needs study.

Two children were studied twice – once when they were in good health and again 3 months later when they had developed bronchopneumonia. They showed complete absorption of vitamin A when they were normal but the absorption dropped considerably when they were ill (Table 4).

Enterohepatic circulation of vitamin A has been demonstrated in the rat (Olson, 1968). But the almost complete absence of label in the faeces of the normal subjects studied here suggests that the amount of vitamin excreted in bile may be negligible in human beings, alternatively there is complete reabsorption of vitamin A excreted in bile.

Table 4. *Absorption of labelled vitamin A in two children at two periods: without and during infection*

(Dose as in Table 1)

Subject no.	% of dose absorbed from the gut	% of the absorbed label excreted in urine
3: Normal	100·0	16·6
With respiratory infection	80·0	21·0
6: Normal	100·0	11·4
With respiratory infection	30·7	24·8

In children with infection, vitamin A was excreted partly as such and partly in the form of degraded products. The break-down products may perhaps have resulted from bacterial action on unabsorbed vitamin A present in the gut.

In children with acute diarrhoea, there was an appreciable decrease in the intestinal absorption of the vitamin. In these children, most of the radioactivity was present in the unsaponifiable fraction of the faeces, which may be taken as reflecting the amount of unabsorbed vitamin A. The defective absorption in these children could be due either to infection or to the excessively rapid passage of the intestinal contents, or to both. It has been reported earlier that, in subjects with steatorrhoea (May & Lowe, 1948) and giardiasis (Katsampes, McCoord & Phillips, 1944), absorption of vitamin A is seriously impaired.

Repeated attacks of respiratory infections and diarrhoea may thus enhance the vitamin requirements by interfering with intestinal absorption of the vitamin. In children whose dietary intakes are low and whose body stores of the vitamin are marginal, this may precipitate clinical manifestations of vitamin A deficiency. In addition to an inadequate dietary intake of vitamin A, a high incidence of repeated attacks of infection may contribute significantly to the wide prevalence of vitamin A deficiency in children.

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