

Studies of Vitamin A Deficiency in Children

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AMONG THE clinical manifestations of malnutrition in Indian children, those attributable to deficiencies of protein and vitamin A are the most widespread. While a great deal of attention has been devoted in recent years to the study of the problem of protein malnutrition, the problem of vitamin A deficiency which is no less important and more easily preventable has not attracted the same interest. It has been our experience (in Coonoor and Hyderabad, South India) that patients with vitamin A deficiency account for 25 to 30 per cent of all cases of clinical malnutrition in children.

Although the clinical manifestations of vitamin A deficiency have been recognized and described, there are still many aspects of the problem which would appear to require elucidation. For example, the frequent association of clinical signs of vitamin A deficiency with kwashiorkor suggests a possible interrelationship between protein malnutrition and vitamin A deficiency. The relationship between dietary intake of vitamin A and carotene, the levels of vitamin A in the serum, and the incidence and severity of clinical manifestations of vitamin A deficiency would seem to merit further investigation. In this paper the results of investigations of these and some other aspects of the problem of vitamin A deficiency in children are presented.

MATERIAL AND METHODS

The material for this investigation consisted of 319 cases of vitamin A deficiency, observed in Coonoor over a five year period (1952 to 1956) and forty-nine cases studied in Hyderabad over an eight month period in 1959. The investigation included the following:

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clinical examination of cases, a survey of dietary intake with special reference to vitamin A and carotene, estimation of serum vitamin A and carotene levels before and after treatment and determination of the *in vitro* destruction of vitamin A by the patients' lysed red blood corpuscles. In addition, data on the incidence of vitamin A deficiency, obtained from the records of the Niloufer Hospital, Hyderabad for the five year period of 1954 to 1958, have also been considered. The assessment of dietary intake was carried out using the oral questionnaire method.

Serum vitamin A and carotene were estimated spectrophotometrically as follows. The absorption at 460 m μ and the difference in absorption at 328 m μ before and after irradiation were taken as measures of the carotene and vitamin A, respectively. Three ml. of serum or plasma was hydrolyzed with alcoholic KOH and extracted with petroleum ether. The layer of petroleum ether was washed and dried under vacuum and taken up in cyclohexane. After the initial readings were taken on the cyclohexane solution, it was irradiated for half an hour and a second measure of absorbance at 328 m μ was taken. The calculations were made as usual, taking the conversion factors into account.

The time required for the irradiation and the lower limit of the method were determined with solutions of pure vitamin A. All the solvents were specially purified and a reagent blank was carried out with every set of samples. It was found that a concentration below 10 I.U. per 100 ml. could not be estimated properly. The correction of Morton and Stubbs gave values in agreement with the aforementioned procedure only in samples of serum which had a high concentration of vitamin A.

The destruction of vitamin A by hemolyzed red blood cells was determined by the following

TABLE I
Age and Sex Incidence of Subjects with Vitamin A Deficiency

City	No. of Patients	Age (per cent)					Sex (per cent)	
		Less than 1 Year	1 to 3 Years	3 to 5 Years	6 to 10 Years	Over 10 Years	Males	Females
Coonoor	319	0.2	17.9	39.0	31.4	11.5	58	42
Hyderabad	551	3.8	26.8	48.7	18.2	2.5	60	40

procedure. The erythrocytes were separated from oxalated blood and washed with saline. Four volumes of water were added and the lysis was completed by freezing and thawing. Five ml. of the lysed solution equivalent to 1 ml. of red blood cells was used. Concentrated emulsion of vitamin A acetate was prepared, according to Pollard and Bieri.¹ It was suitably diluted on the day of estimation so that 0.5 ml. of emulsion would be equivalent to about 25 I.U. of vitamin A. The lysed cells and 0.5 ml. of the emulsion were incubated at 37° c. Alcoholic KOH was added at the end of fifteen minutes. Vitamin A was extracted and estimated with antimony trichloride solution. The vitamin A concentration in the emulsion was checked at the time of each estimation. The reduction in the vitamin A content of the incubated sample was taken as the amount of vitamin A destroyed and expressed as a per cent of the actual amount incubated.

The estimations of serum vitamin A and carotene and the determination of the *in vitro* destruction of vitamin A by the patient's lysed red blood cells were carried out in cases of vitamin A deficiency studied in Hyderabad. The estimations were repeated after treatment in a number of cases. For purposes of comparison, similar estimations were also carried out in apparently normal children of the same age group and socio-economic status in Hyderabad and in children with kwashiorkor who did not show clinical signs of vitamin A deficiency. Data concerning the different groups of children from whom these estimations were made, their clinical condition and the plan of treatment adopted are included in this report. (See Table III.)

The treatment in cases of kwashiorkor without vitamin A deficiency signs (Group 2) consisted of the administration of a high protein diet without a vitamin A supplement (the protein being almost solely derived from skim milk to which no vitamin A was added). Subjects with kwashiorkor and with signs of vitamin A deficiency (Group 4) received a high protein diet and, in addition, supplements of vitamin A daily. The vitamin A deficiency observed in nine of the seventeen children in this group was of the severe type involving the cornea, necessitating parenteral (in preference to oral) administration of vitamin A. These patients received 200,000 U. of vitamin A parenterally each day for eight to ten days; they subsequently were given 90,000 U. of vitamin A orally each day for approximately a fortnight. The remaining eight persons in this group, showing only conjunctival signs of vitamin A deficiency, were treated orally. Thirteen of the subjects with vitamin A deficiency and without kwashiorkor in Group 3a exhibited only conjunctival manifestations while four had keratomalacia. Patients with conjunctival manifestations received oral treatment, while those with keratomalacia received 200,000 U. parenterally. All children in Group 3b presented conjunctival manifestations, only.

All patients studied were afebrile at the time of the investigation.

Incidence and Clinical Features

The age and sex incidence of the children with vitamin A deficiency are indicated in Table I, and the clinical features of the subjects studied (319 in Coonoor and forty-nine in Hyderabad)

TABLE II
Main Clinical Features of Vitamin A Deficiency in Children

City	Total Number of Patients	Clinical Features			
		Conjunctival Lesion Only	Night Blindness Only	Conjunctival Lesion and Night Blindness	Keratomalacia
Coonoor	319	113	85	98	23
Hyderabad	49	14	0	2	33

are given in Table II. The maximal incidence was observed in the age period between three and five years. Nearly 60 per cent of the subjects were boys and 40 per cent were girls. However, this difference in sex incidence may reflect the general attendance of the two sexes in the clinic and may possibly be related to the tendency of poor women to seek medical advice more promptly and frequently for their boys than for their girls. It may thus have no etiologic significance.

Of the 319 children observed in Coonoor, only twenty-three showed corneal involvement (7.2 per cent). In the great majority of patients, the signs of hypovitaminosis A were of the milder type, involving only the conjunctiva. Bitot's spots were encountered in 102 of the 211 subjects showing conjunctival manifestations, and were invariably bilateral and situated on the lateral side of the cornea. The remaining children showed conjunctival xerosis characterized by dryness, discoloration and wrinkling of the bulbar conjunctiva without Bitot's spots.

An interesting feature was the lack of association between the presence of night blindness and the incidence of visible signs in the eye of vitamin A deficiency. Eighty-five children had night blindness without any other clinical manifestation of vitamin A deficiency, for the conjunctiva and cornea were clear and normal. In 113 subjects showing conjunctival lesions, no history of night blindness could be obtained, even after leading questions were posed. Keratomalacia observed in twenty-three patients was invariably bilateral with one eye being more affected than the other, and included lesions conforming to both the types

described by Oomen² (viz., "multiple erosions of the conjunctiva leading to mummification of the cornea but without loss of general shape of the cornea" and "colliquative necrosis of the whole cornea leading to shrinkage of the eye ball"). From the etiologic viewpoint and from the responses obtained through treatment, there seemed to be no clear-cut distinction between these two types as they appeared to be merely clinical variations related to the speed and the severity of the same disease.

Of the forty-nine patients studied in Hyderabad, thirty-three had the severe type of vitamin A deficiency involving the cornea. In only sixteen cases were the lesions of the conjunctival type. The preponderance of corneal involvement in this particular investigation is in contrast to the low incidence (7.2 per cent) of keratomalacia found in Coonoor. It was quite clear that the vitamin A deficiencies in the children of Hyderabad were of a more severe type than those observed in the children of Coonoor. The age of the subject did not appear to be a factor in determining whether the site of the lesion occurred on the conjunctiva or the cornea. A possible objection to the assessment of the incidence of keratomalacia, in relation to the total incidence of vitamin A deficiency, purely on the basis of hospital admissions might be that only persons with severe vitamin A deficiency may be admitted, while those with the milder conjunctival type may have received treatment as outpatients. To obviate this possible objection, the incidence of manifestations of vitamin A deficiency in a series of patients admitted to the hospital in Hyderabad for kwashiorkor was compared with a series of patients with kwashiorkor

TABLE III
Serum Carotene, Vitamin A and the *In Vitro* Destruction of Vitamin A by the Red Blood Cells in Different Groups of Children.

Group No.	Clinical Condition	Treatment	Serum Carotene* (ratio/100 ml.)		Serum Vitamin A (I.U./100 ml.)*				<i>In vitro</i> Destruction of Vitamin A* (per cent)	
			Before Treatment	After Treatment	Before Treatment †		After Treatment †		Before Treatment	After Treatment
					No. Below 10 I.U.	Mean	No. Below 10 I.U.	Mean		
1	Apparently normal	...	50 (6)	...	0	80 (6)	28 (4)	...
2	Kwashiorkor only	High protein diet with no vitamin A	22 (13)	19 (7)	5	52 (9)	0	71 (7)	28 (5)	...
3a	Vitamin A deficiency only	Vitamin A	18 (13)	26 (11)	6	32 (7)	0	102 (11)	52 (8)	30 (3)
3b	Vitamin A deficiency only	High protein diet with no vitamin A	12 (4)	11 (3)	2	45 (2)	2	59 (1) ‡	62 (4)	79 (3)
4	Kwashiorkor with vitamin A deficiency	High protein diet with vitamin A	13 (14)	19 (9)	12	17 (2)	0	74 (10)	45 (4)	34 (2)

* The values given are the mean values of the number of samples indicated in parentheses. The number of patients after treatment includes some patients for which initial values were not available.

† The number of samples having a concentration of less than 10 I.U. have been indicated in this column, and the mean given in the next column is the average of values above 10 I.U.

‡ This particular sample had a concentration of 65 I.U. of vitamin A/100 ml. serum initially.

observed in Coonoor. The over-all incidence of vitamin A deficiency in the patients with kwashiorkor in both Hyderabad and Coonoor was between 32 to 36 per cent. However, while keratomalacia accounted for only 10 per cent of the children with vitamin A deficiency complicated with kwashiorkor in Coonoor, it accounted for 62 per cent, in Hyderabad. This indicates that the observed differences between the incidence of children with keratomalacia in Hyderabad and in Coonoor were real.

An examination of the monthly incidence of cases for a five year period failed to reveal any definite seasonal trend in both places. There was no correlation between the incidence of cases of vitamin A deficiency and of respiratory or alimentary disorders.

The Dietary Situation

An assessment of the dietary intake of

carotene and vitamin A was carried out in a group of children with vitamin A deficiency in Hyderabad. A similar survey was also carried out using a control group of children of the same age composition and socio-economic status who did not show any signs of vitamin A deficiency. It was found that the vitamin A and carotene intakes of both the groups were considerably lower (250 to 300 I.U. of precursor and 100 to 150 I.U. of preformed vitamin A) than the recommended quantities. There was no significant difference between the control and vitamin A deficiency groups regarding carotene and vitamin A intake. It had been reported earlier that the intakes of carotene and vitamin A of patients with kwashiorkor in Hyderabad were not lower than those in Coonoor in spite of the fact that the incidence of keratomalacia was much higher in the former.³

It was apparent from these findings that

while vitamin A deficiency signs were always associated with a low dietary intake of carotene and vitamin A, the reverse was not true. In a number of patients subsisting on low dietary intakes of carotene and vitamin A, no clinical signs of vitamin A deficiency could be detected. A closer examination of the diets of children with kwashiorkor also failed to reveal any striking difference (with regard to dietary vitamin A and carotene intake) between patients with and those without complicating vitamin A deficiency signs.

Serum Vitamin A and Carotene Levels

The values for levels of vitamin A and carotene in the serum of children in the four groups investigated in Hyderabad are included in Table III. The levels of vitamin A in the serum of the apparently normal children (Group 1) were lower than the average values reported for normal children in other parts of the world.^{2,4} This finding was not unexpected, for although the children investigated in this group were free from disease, they were drawn from the poor socio-economic group subsisting on unsatisfactory diets. As expected, the subjects with vitamin A deficiencies (Group 3) exhibited considerably lower levels of serum vitamin A than did those of Group 1. It was interesting that patients with kwashiorkor with no signs of vitamin A deficiency (Group 2) also exhibited levels of serum vitamin A which were significantly lower than those observed in the children in Group 1. The lowest levels of serum vitamin A were observed in the subjects exhibiting signs of both kwashiorkor and vitamin A deficiency (Group 4). The values for serum carotene were uniformly low in all the groups.

After treatment with vitamin A, a marked increase in the levels of vitamin A in the serum was observed in all subjects with vitamin A deficiencies. A striking observation was made of the children with kwashiorkor who exhibited low levels of serum vitamin A. Treatment of these children with a high protein diet containing *no* vitamin A supplement brought about a significant increase in the levels of vitamin A in the serum. It was apparent from these observations that protein malnutri-

tion can bring about a significant lowering of serum vitamin A, and treatment with a high protein diet without vitamin A supplementation can correct this.

In Vitro Destruction of Vitamin A

In apparently normal children studied in this investigation (Group 1), the *in vitro* destruction of vitamin A by the lysed red cells was approximately 28 per cent. Subjects with kwashiorkor (Group 2) did not significantly differ from the normal children (Group 1) in this regard. However, in children with vitamin A deficiencies with or without kwashiorkor (Groups 3 and 4), the extent of *in vitro* destruction of vitamin A appeared to be definitely of a much higher order than that of the normal subjects (Group 1). However, there was no difference between Groups 3 and 4 which again demonstrated that the presence of kwashiorkor did not influence this function.

After treatment with vitamin A, it was noticed that the abnormally high destruction, *in vitro*, of vitamin A was lowered in regard to patients in groups 3 and 4 with vitamin A deficiencies.

COMMENTS

Dietary Intake

An important observation made in this study was that while the dietary intakes of vitamin A and carotene of children with vitamin A deficiencies were low, they were not significantly lower than those of other children of the same socio-economic group who did not show signs of vitamin A deficiency. This would suggest that in the development of vitamin A deficiency, factors other than the actual diets of the children at the time of the investigation also have to be considered. Although it is true that in a number of patients the onset of clinical signs of vitamin A deficiency was preceded by episodes of fevers, infections and alimentary disorders, such episodes were apparently no more frequent in children showing signs of vitamin A deficiency than in those of the same socio-economic group not suffering from this deficiency. The time at which supplementary feeding was started



may be an important factor in this connection. Mothers in Coonoor started the supplementary feeding of their infants by the sixth month, while in Hyderabad supplementary feeding was initiated only after the end of the first year.^{3,5} This would indicate that, although at the time of investigation the intake of vitamin A and carotene by the children in both places was nearly similar, the children of Hyderabad had been on a vitamin A deficient diet for a relatively longer period of time. This might partly account for the higher incidence of severe types of vitamin A deficiency in Hyderabad. The vitamin A content of the breast milk of poor Indian women in Coonoor has been shown to be about 70 I.U./100 ml.⁶ The vitamin A content of the breast milk of poor mothers in Hyderabad is being determined at present. The preliminary indications are that the content of vitamin A in the latter is considerably lower than that observed in the women in Coonoor. Even assuming that the vitamin A content of milk in Hyderabad mothers is the same as in the Coonoor mothers and that the output of breast milk in the former women is as high as 600 ml. throughout the first year of motherhood, breast milk alone would provide only 400 I.U. of vitamin A to the infants.

Another important factor which might explain the lack of a direct relationship between the dietary intake of vitamin A by the children and the incidence of clinical vitamin A deficiency among them is the possible variation in the hepatic storage of vitamin A during their fetal periods. In human adults with presumably adequate hepatic storage of vitamin A, dietary deprivation of vitamin A, extending over several months, was necessary to produce significant depression in the levels of vitamin A in the serum.⁷ The livers of newborn infants, whose mothers' intake of vitamin A is presumably adequate, have been found to contain considerable amounts of vitamin A.⁸ A survey* of the diets of pregnant mothers in Hyderabad revealed gross deficiencies of vitamin A and carotene. The estimation of vita-

min A in the livers of stillborn infants and of those dying during the neonatal period (which is at present being attempted) will provide further information on this subject.

It may, however, be appropriate to emphasize the fact that no case of vitamin A deficiency was encountered, in this study, in a child with a really satisfactory intake of vitamin A and carotene.

Night Blindness and Other Signs

A lack of association between the incidence of night blindness and other ocular signs of vitamin A deficiency was observed in our study. The incidence of night blindness may be modified by such factors as the degree of exposure to sunlight,^{9,10} the degree of associated anemia¹¹ and possibly the nutritional status with regard to riboflavin¹² and vitamin C.^{13,14}

Protein Malnutrition and Vitamin A Deficiency

The question of the relationship between protein malnutrition and vitamin A deficiency has attracted some attention in recent years.² In our investigation, it was observed that serum vitamin A levels were significantly lowered in patients with kwashiorkor who showed no clinical evidence of vitamin A deficiency; and furthermore, that treatment with a high protein diet without a vitamin A supplement brought about an increase in the levels of vitamin A in the serum in these patients. It was also found that subjects with a vitamin A deficiency complicated with kwashiorkor had significantly lower levels of serum vitamin A than did those uncomplicated without kwashiorkor, or vitamin A deficiency. These observations might suggest that protein malnutrition might aggravate vitamin A deficiency. Arroyave et al.¹⁵ found that the absorption of vitamin A was impaired in patients with kwashiorkor. The increase in the levels of vitamin A in the serum, brought about in subjects with kwashiorkor by a high protein diet (in this study), indicate that the effect of protein malnutrition may be an impairment of the mobilization of vitamin A stored in the liver. It was demonstrated that when there is no manifest protein malnutrition in a child deficient in vitamin A,

* This survey, taken by Dr. X. X. Kalpakum, has not yet been published.

the administration of a high protein diet does not result in an increase in the levels of vitamin A in the serum. The significance of lower levels of vitamin A in the serum of patients with kwashiorkor requires further study.

Contrary to the suggestion that protein depletion may aggravate vitamin A deficiency, Jagannathan¹⁶ found that increasing the amount of protein in the diet beyond a certain point had a hastening effect on the depletion of the stores of vitamin A in experimental animals. He also observed an inverse relationship between growth in young rats and hepatic storage of vitamin A. The work of McClaren¹⁷ indicated that protein depletion actually delayed the development of signs of vitamin A deficiency in rats fed diets deficient in vitamin A. During our study, it was observed that six children with kwashiorkor, who had no signs of vitamin A deficiency on admission, developed conjunctival signs of vitamin A deficiency after a few weeks of treatment with a high protein diet. We have frequently observed patients with severe keratomalacia without any evidence of kwashiorkor and patients with kwashiorkor with absolutely no clinical evidence of vitamin A deficiency.

The apparently contradictory observations of the relationship between protein depletion and vitamin A deficiency can be explained if it is recognized that protein depletion may, on the one hand, aggravate vitamin A deficiency by interfering with the absorption of vitamin A and possibly with the mobilization of vitamin A from the liver, and, on the other hand, mitigate vitamin A deficiency by sparing the tissue requirement of vitamin A by inducing growth retardation. The degree of protein depletion may well determine the direction of the net effect.

Serum Vitamin A and Carotene Levels

In the subjects with vitamin A deficiency, both the serum vitamin A levels as well as the serum carotene levels were low. It would thus appear that defective conversion of carotene into vitamin A was not a factor in the development of this disease. The lowest value for the levels of vitamin A in the serum observed in an apparently normal child (without any

evidence of vitamin A deficiency) was 45 I.U./100 ml. The highest level of vitamin A in the serum observed in the series of patients investigated with uncomplicated vitamin A deficiencies was 65 I.U./100 ml.

We have observed that the state of protein nutrition may also be an important factor in determining the levels of vitamin A in the serum in a malnourished population. The results of the present investigation do not permit any conclusions as to the critical level of serum vitamin A values below which it could be considered to be of definite pathologic significance. However, it was apparent that in children with vitamin A deficiency but without complicating kwashiorkor, values for serum vitamin A below 50 I.U. were the general rule.

In Vitro Destruction of Vitamin A

The work of Kon et al.¹⁸ indicated the possible presence of factors which cause destruction of vitamin A in rabbits and rats. Pollard and Bieri¹ showed that the lysed red blood cells of rats, *in vitro*, were most active in destroying vitamin A. In our study, we observed that the lysed red cells from the blood of patients with vitamin A deficiencies were much more potent in bringing about *in vitro* destruction of vitamin A than were those of normal subjects. Furthermore, we were able to correct this abnormality by treating these patients with vitamin A. Additional investigation is necessary in order to decide to what extent this observation is applicable to the *in vivo* state. The significance of this observation from the point of view of the pathogenesis of manifestations of vitamin A deficiency remains to be elucidated. The results obtained through treatment indicate that the capacity for increased *in vitro* destruction of vitamin A by the lysed red blood cells of patients with vitamin A deficiency is the result rather than the cause of vitamin A deficiency.

SUMMARY

The clinical features of 319 children with vitamin A deficiency observed in Coonoor and forty-nine children studied in Hyderabad have been discussed in detail. Estimations of the contents of vitamin A and carotene in the serum



and the *in vitro* destruction of vitamin A by lysed red blood cells were carried out.

There was a lack of association between the incidence of night blindness and other ocular signs of vitamin A deficiency. Although signs of vitamin A deficiency were always associated with a low dietary intake of carotene and vitamin A, the reverse did not always occur. The proportion of patients with vitamin A deficiency who had keratomalacia was much greater in Hyderabad than in Coonoor.

Levels of vitamin A and carotene in the serum were low in all children with vitamin A deficiency. Levels of vitamin A in the serum of subjects with kwashiorkor but without clinical signs of vitamin A deficiency were also found to be significantly lower than the levels in apparently normal children. Treatment with a high protein diet without vitamin A supplementation brought about a significant increase in the levels of vitamin A in the serum in the latter.

The *in vitro* destruction of vitamin A by lysed red blood cells of children suffering from vitamin A deficiency appeared to be higher than that of the normal controls. Treatment with vitamin A lowered this abnormal *in vitro* destruction.

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