BIOCHEMICAL EVALUATION OF THE NUTRITION STATUS OF URBAN PRIMARY SCHOOL CHILDREN : VITAMIN-A STATUS

J. P. DU PLESSIS AND D. J. DE LANGE, *Division of Physiological Chemistry* AND M. L. NESER, *Division of Field Studies, National Nutrition Research Institute, Council for Scientific and Industrial Research, Pretoria*

In a world-wide survey of the incidence of xerophthalmia carried out by the World Health Organization in 1962/63, the data collected by Escapini in Latin America, by Oomen in Asia and by McLaren in Africa, showed that xerophthalmia due to severe vitamin-A deficiency, remained the most important cause of blindness in the young child.[1] The results of this survey indicated that tens of thousands of children were being blinded annually by xerophthalmia, especially in Indonesia, South India, Ceylon, East Pakistan, the Philippines, Vietnam, the Central American Republics, Haiti, North East Brazil, parts of North and Central Africa including Egypt and the Sudan, Jordan, Syria and Iraq.

According to McLaren[1] the incidence of blindness due to xerophthalmia in institutions for the blind is deceptively low in relation to the over-all incidence. In a study on children with protein malnutrition in Jordan, it was found that when xerophthalmia was a complicating factor the death rate was 80%, whereas it was only 15% in a comparable group without evidence of vitamin-A deficiency. The deaths in the group with xerophthalmia were due to intercurrent infection, indicating that there is an impaired resistance to infection in such children which causes a large proportion of premature deaths and thus reduces the numbers of xerophthalmia victims who survive to enter institutions.

The extent and character of the pathological changes produced by vitamin-A deficiency will depend on the duration of the deficiency, the quality of the diet, the bacterial environment of the affected subjects, their sex and the extent to which they are subjected to stress such as variations in temperature.[2] According to Moore,[3] three basic defects are produced by vitamin-A deficiency:

(*i*) Defective dark adaptation due to a deficiency of the aldehyde form which is required for the production of rhodopsin.

(*ii*) Keratinization of the mucous membranes in various parts of the body.

(*iii*) Defective skeletal architecture due to a vitamin-A deficiency during growth.

Despite the rich sources of provitamin A available in carrots, maize, sweet potatoes, pawpaws, mangoes, dates and other vegetable foods which are grown in abundance on this continent, vitamin-A deficiency is apparently prevalent in many parts of Africa.[4,5] It is self-evident, therefore, that we should be in a position to recognize latent as well as overt vitamin-A deficiency so that preventive measures can be instituted at an early stage before irreversible damage has been done.

From the point of view of practicability, there are currently only two biochemical tests available for the evaluation of vitamin-A status in population groups. These are tests which measure the vitamin A and carotene concentrations in the serum. Had it been possible to determine liver reserves of vitamin A from biopsy specimens, the evaluation of vitamin-A status would have presented no great difficulty, but liver biopsy is not a procedure that can be adopted for routine use in population studies. The present study is therefore based only on serum vitamin A and carotene determinations, which have long been used as criteria of the vitamin-A status of populations and individuals.[6]

With the dual object of evaluating biochemical methods for the assessment of nutritional status and assessing the state of nutrition of the 4 main racial groups in Pretoria, the National Nutrition Research Institute carried out nutrition status surveys on the following population groups:

(*a*) 560 White school children aged 7 - 11 years (April - June 1962).

(*b*) 585 Bantu school children aged 7 - 15 years (April - June 1963).

(*c*) 450 Coloured and 407 Indian school children aged 7 - 15 years (April - June 1964).

(*d*) 248 White school children aged 12 - 15 years (April - May 1965).

Since the survey on the older White children was carried out 3 years later than the one on the younger children, it would not have been correct from a statistical point of view to treat the data for the two surveys as an unbroken sequence of observations. For the purpose of statistical analysis, therefore, the children of 7 - 11 years and those of 12 - 15 years have been treated as two independent groups.

MATERIAL AND METHODS

The surveys were based on statistically representative samples drawn at random from the 4 population groups. The statistical planning of the surveys has been fully described by Fellingham[7] and the sample and population sizes are given in detail in previous papers by Du Plessis *et al.*[8,9]

Vitamin A and carotene were determined simultaneously according to the micromethod of Bessey *et al.*[10] The method was slightly modified in our laboratory in so far as an additional centrifugation was introduced. It needs to be stressed, however, that this method measures the total carotenoids in the serum, some of which may have no vitamin-A activity, and that this detracts from the specificity of the test as a criterion of nutrition status.

RESULTS AND DISCUSSION

The biochemical results are given in the form of frequency distribution curves (Figs. 1 - 4). The 5th, 10th, 50th, 90th and 95th percentiles are also given in these figures. The results of the analysis of variance in respect of race, sex and age and of the multiple comparisons test in respect of race are given in Table I. A 5% level of significance (two-tailed) was applied throughout.

A comparison of the vitamin-A levels of the four racial groups in the two age ranges (Figs. 1 and 2) indicates that in both the younger and the older age-groups appreciably higher results were obtained for White children than for those of any of the other three racial groups. The statistical analysis of variance showed that the results were not influenced by age or sex in either of the two age-groups. The multiple comparisons test in respect of race (see Table I)

TABLE I. THREE-WAY ANALYSIS OF VARIANCE AND MULTIPLE COMPARISONS TEST FOR DIFFERENCES DUE TO RACE, SEX AND AGE*

		Race		Sex	Age
Variable	*Age-group*	*Analysis of variance*	*Multiple comparisons*†	*Analysis of variance*	*Analysis of variance*
Vitamin A	7-11 years	P<·1%	WBCI	P> 5%	P>5%
Carotene	7-11 years	P<·1%	WBCI	P<0·1%	P>5%
Vitamin A	12-15 years	P<·1%	WBCI	P> 5%	P>5 %
Carotene	12-15 years	P<·1%	WBCI	P> 5%	P>5%

*P values of 5% or less indicate a significant difference.

†W = White, B = Bantu, C = Coloured, I = Indian. The convention has been followed of underlining with a common line all groups which did not differ significantly at a 5% level.

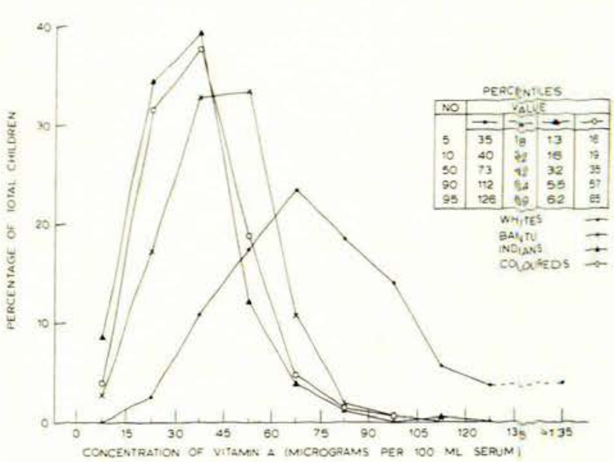

Fig. 1. Frequency distribution of serum vitamin-A values in Pretoria children of 7 - 11 years.

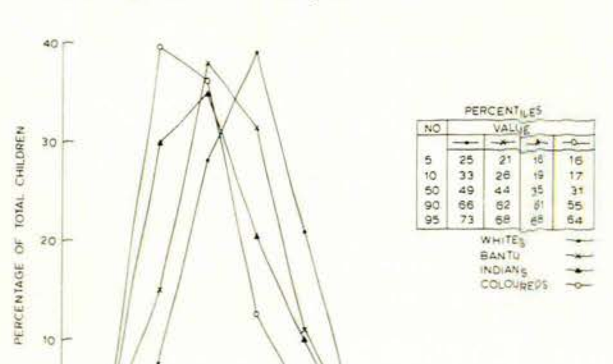

Fig. 2. Frequency distribution of serum vitamin-A values in Pretoria children of 12 - 15 years.

showed that with the exception of Bantu and Coloured children in the younger age-group and Coloured and Indian children in both age-groups, all races in each of the two age-groups differed significantly from one another.

The trends shown by the carotene determinations were on the whole similar to those indicated by the vitamin-A determinations. Substantially higher values were again obtained for the White children than for any of the other 3 racial groups (Figs. 3 and 4). According to the analysis of variance, age had no influence on the serum carotene values in either of the two age-groups. The values were, however, influenced by sex in the children of 7 - 11 years, though not in the older age-group. The multiple comparisons test in respect of race (Table I) showed that there were no significant differences between Bantu, Coloured and Indian children in the younger age-group or between Bantu and Coloured children in the older group, all other differences, however, being significant.

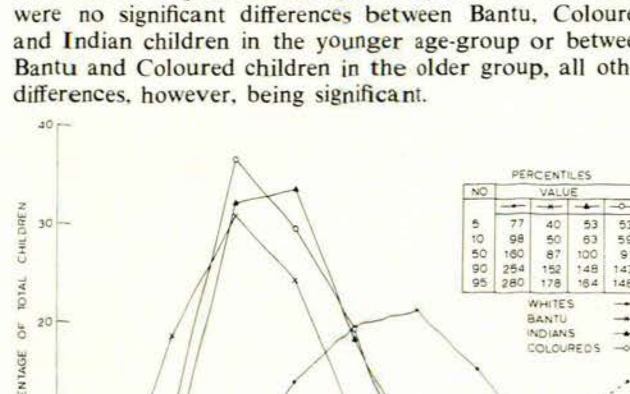

Fig. 3. Frequency distribution of serum carotene values in Pretoria children of 7 - 11 years.

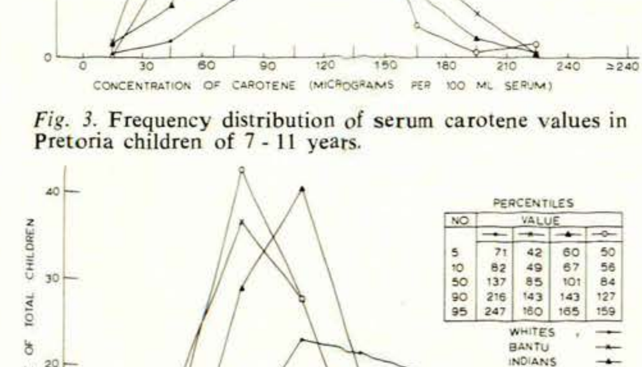

Fig. 4. Frequency distribution of serum carotene values in Pretoria children of 12 - 15 years.

Before attempting to interpret the findings for the various groups in terms of nutrition status, it is necessary to consider the main factors responsible for the concentration of vitamin A and carotene present in the serum, which are the following:

The Vitamin A and Carotene Content of the Diet

The fact that there is a relationship between serum vitamin A and carotene levels and the dietary intake of these two nutrients forms the basis of the current practice of evaluating the adequacy of the diet in respect of these nutrients in terms of the concentrations present in the serum.[30] This relationship does not necessarily consist, however, in a straightforward linear correlation between the serum levels and the habitual dietary intake, and must be seen in its correct perspective if the implications of the serum values are to be realistically assessed.

Since surplus vitamin A is stored in the liver, the reserves there available can be drawn upon when the dietary intake is deficient and the serum concentration maintained at a level that is physiologically adequate, though this level may be considerably lower than those normally maintained on higher intakes.[11] This interpretation of the body's response to the stress imposed by a deficiency of vitamin A in the diet is in keeping with the findings of Hume and Krebs.[11] It is only when the liver reserves are nearing depletion, which may take several months or even years,[11] that a deficiency of vitamin A and its precursors in the diet will be reflected by a substantial drop in serum vitamin A. The presence of vitamin-A reserves in the liver which can be mobilized when the dietary intake is deficient is presumably the explanation of the lack of correlation reported in the literature between serum vitamin-A levels and recent dietary intakes.[12]

The fact that serum vitamin A can be maintained at lower levels in some populations than in others without the development of symptoms pathognomonic of vitamin-A deficiency, as is shown by the results of the Pretoria surveys,* suggests that the body is capable of adapting itself to lower intakes and making more economic use of its vitamin-A resources than it is called upon to do when these are freely available.

It is generally accepted that carotene as such has no active physiological function in the body, and can neither be used nor stored unless converted to vitamin A, of which it is a precursor. Not all forms are convertible to vitamin A, however, and serum levels can therefore be expected to rise when there is a high intake of inactive carotenoids. The fact that serum carotene levels, like vitamin-A levels, need not show any correlation with the recent dietary intakes[12] suggests that the body cannot deal promptly with ingested carotene and effect its rapid withdrawal from the circulation.

Both vitamin A and carotene levels in the serum can be expected to rise after the ingestion of significant amounts in food or vitamin concentrates. This factor need not present any difficulty in population studies, however, if care is taken to ensure that the blood specimens on which the serum evaluations are based are taken at comparable times. The influence of season on the dietary intake of vitamin A and carotene was excluded as far as possible by carrying out all 5 surveys at the same time of year, namely during the April - June school term.

Although it is obvious, therefore, that neither the vitamin A nor the carotene content of the serum will necessarily at all times be a reliable index of either the recent or the habitual intake of an individual or a group, the values obtained on comparable specimens in groups stabilized on their current diet (i.e. in the sense that continued subsistence on the same diet will produce no significant change in the serum values) can be expected to bear a definite relationship to the normal dietary intake.

*The clinical findings for the Pretoria surveys will be published at a later date.

Age and Sex

According to Leitner *et al.*,[13] serum vitamin-A levels are usually higher in men than in women and increase slightly with advancing age in both sexes. Serum carotene levels, on the other hand, were found by them to be slightly higher in women than in men and to show little increase with age. These findings were subsequently confirmed by the ICNND[14] on the basis of their West Indies survey.

The results of the present study are not strictly comparable with those of Leitner and the ICNND because of the age difference in the populations studied, but our results at least partly confirm their conclusions. In our surveys higher mean values for carotene were also found for girls (122 and 111 μg./100 ml. in the younger and older groups respectively) than for boys (respectively 109 and 105 μg./100 ml. in the younger and older groups), but no significant differences attributable to age. The sex difference was statistically significant in the children of 7 - 11 years ($P<0.1\%$), but not in the older group ($P>5\%$). In the case of vitamin A our findings disclosed no evidence of any influence attributable either to age or sex.

The absence of any detectable influence of age on the serum vitamin A and carotene concentrations of the Pretoria children and the similarity between our values for White children and the values reported in the literature for adults of comparable socio-economic status[11,14] suggest that 'adult' serum vitamin A and carotene concentrations have already been attained by the age of 7 years. Since the effect of sex on the carotene values was too small to justify the application of separate standards to girls, nothing has emerged from the results of our surveys to invalidate the application to all children of 7 years and over of the criteria defined by the ICNND[15] for the evaluation of vitamin A and carotene status in adult males.

Serum Protein Levels

Serum vitamin A is transported in the form of a protein complex and the maintenance of an adequate concentration of vitamin A in the serum will clearly, therefore, be dependent on the presence of adequate quantities of the relevant protein fraction. According to Krinsky *et al.*[16] and Garbers *et al.*[17] the transportation of vitamin A is effected by the α_1-globulin fraction, while Erwin *et al.*[18] and Friend *et al.*[19] attribute this function to albumin. Whatever the protein fraction responsible, however, protein deficiency can safely be discounted as a cause of low vitamin-A levels in the Pretoria children, for no evidence was found in any of the 4 racial groups[9,20] of deficiencies capable of influencing the vitamin-A carrying capacity of the serum.

The ranges for adult males proposed by the ICNND[15] for the nutritional evaluation of serum vitamin-A levels were based on the fact that the values for normal populations usually fall between 30 and 50 μg. per 100 ml. serum,[21] on a controlled intake study done by the British Medical Council[12] and on work aimed at discovering the serum levels at which clinical symptoms begin to appear.[22] The scale proposed by the ICNND is considerably less stringent than those suggested by Bessey and Lowry[23] and by Sinclair,[24] but agrees well with those of Williams *et al.*[25] and Clayton *et al.*[26]

An evaluation of the serum vitamin-A levels of the Pretoria children in terms of the ICNND standards is given in Tables II and III for children of 7 - 11 and 12 - 15 years respectively. According to the ICNND, the presence of 5% or more subjects with serum vitamin-A values below 10 μg./100 ml., or 15% with values below 20 μg./100 ml., is indicative of vitamin-A deficiency in the population as a whole.

TABLE II. DISTRIBUTION OF SERUM VITAMIN-A VALUES IN CHILDREN OF 7–11 YEARS ACCORDING TO THE ICNND STANDARDS

Category	*White children** %	*Bantu children** %	*Indian children** %	*Coloured children** %
Deficient <10 μg./100 ml.	0·2	0·3	2·4	1·3
Low 10–19 μg./100 ml.	0	5·6	17·0	8·8
Acceptable 20–50 μg./100 ml.	19·8	63·0	67·0	72·8
High >50 μg./100 ml.	80·0	31·1	13·6	17·1

*Total numbers of children investigated are given on page 1093.

TABLE III. DISTRIBUTION OF SERUM VITAMIN-A VALUES IN CHILDREN OF 12–15 YEARS ACCORDING TO THE ICNND STANDARDS

Category	*White children** %	*Bantu children** %	*Indian children** %	*Coloured children** %
Deficient	0	0	0	0·6
Low	2·0	0·8	9·4	14·9
Acceptable	49·6	40·7	46·2	52·3
High	48·4	58·6	43·1	32·2

*Total numbers of children investigated are given on page 1093.

Application of the ICNND criteria to the serum vitamin-A value obtained in the Pretoria surveys (Tables II and III) indicates that the White and Bantu children showed virtually no evidence of vitamin-A deficiency in either age-group. A small proportion (5·6%) of the Bantu children in the younger age-group fell into the 'low' category, and the percentage with values in the 'high' range was much lower in these children than in the White children of corresponding age. In the children of 12 - 15 years, however, the distribution of the values was practically identical in the 2 racial groups. The vitamin-A status of White and Bantu school children would therefore appear to be highly satisfactory in 3 of the 4 groups studied, namely the two White groups and the older Bantu group, and somewhat lower, but nevertheless well within the acceptable range, in the Bantu children of 7 - 11 years.

The findings for the Coloured and Indian children are less satisfactory, for in both age-groups a significant proportion of the values for these 2 racial groups fell into the 'low' (and therefore potentially deficient) range, the highest figure being that for the younger Indian children (17%) and the next highest (14%) that for the older Coloured children. A few of the Coloured and Indian children in the younger age-group (2·4 and 1·3% respectively) fell into the 'deficient' category, and the percentages in the 'high' category were much lower in these groups than in any of the others. It would appear, therefore, that a measure of latent vitamin-A deficiency exists among Coloured and Indian school children in Pretoria, the incidence being highest among Indian children of 7 - 11 years. Whether such a latent deficiency is actually in itself harmful is a question that cannot at present be satisfactorily answered, but there is reason to suspect that it might impair the resistance of the body to infection and other forms of stress.[1]

The results obtained by applying the ICNND standards for serum carotene to the present findings are presented in Tables IV and V.

TABLE IV. DISTRIBUTION OF SERUM CAROTENE VALUES IN CHILDREN OF 7–11 YEARS ACCORDING TO THE ICNND STANDARDS

Category	*White children** %	*Bantu children** %	*Indian children** %	*Coloured children** %
Deficient <20 μg./100 ml.	0·2	0·3	0·5	0
Low 20–39 μg./100 ml.	0·2	4·0	1·9	0·9
Acceptable 40–100 μg./100 ml.	9·9	56·5	48·1	60·1
High >100 μg./100 ml.	89·7	39·1	49·5	39·0

*Total numbers of children investigated are given on page 1093.

TABLE V. DISTRIBUTION OF SERUM CAROTENE VALUES IN CHILDREN OF 12–15 YEARS ACCORDING TO THE ICNND STANDARDS

Category	*White children** %	*Bantu children** %	*Indian children** %	*Coloured children** %
Deficient	0	0	0	0
Low	0	3·8	0·6	1·1
Acceptable	21·8	63·5	47·5	69·0
High	78·2	32·7	51·9	29·9

*Total numbers of children investigated are given on page 1093.

The highest percentages of 'low' values were found in the Bantu children (4·0 and 3·8% in the younger and older age-groups respectively) while in both age-groups, but especially in the younger children, the percentages of values in the 'high' range were much higher in the White than in any of the non-White groups. Of the latter, the highest over-all values were found in the Indian children of 12 - 15 years, but it cannot be said that there was any significant evidence of even a latent carotene deficiency in any of the 4 racial groups.

The question of the latent vitamin-A deficiency found among the Indian and Coloured children deserves consideration from the point of view of the absence of any significant degree of carotene deficiency among these children. As previously indicated, however, an adequate concentration of carotene in the serum is not necessarily a guarantee of an adequate vitamin-A status in the individual, and the apparent adequacy of the serum carotene levels in the Indian and Coloured children does not, therefore, exclude the possibility that a latent deficiency of vitamin A was present in these groups.

SUMMARY

Nutrition status surveys which included the investigation of vitamin-A status were conducted on school children of the four main racial groups between the ages of 7 - 15 years in Pretoria.

The results of these surveys confirmed previous reports that serum carotene values are higher in males than in females. In contrast to the findings of certain other investigators, however, no influence attributable to age was demonstrable in our findings.

The results of these surveys showed no sign of any significant deficiency of vitamin A in White and Bantu children. There were indications, however, that a significant proportion (from 9 to 17%) of the Indian and Coloured children might be suffering from a latent deficiency of vitamin A. This finding is not invalidated by the absence of an accompanying deficiency of carotene in the affected groups.

The following persons were responsible for obtaining the material on which this paper is based: Dr. J. F. Potgieter, Head of Field Studies, NNRI, who was responsible for the organizational planning and practical execution of the field work involved in the 5 Pretoria surveys; Prof. D. J. Stoker, University of Pretoria, who acted in an advisory capacity in the statistical planning of the original survey on the White primary school children; Mr. S. A. Fellingham, of the NRIMS, who planned the first survey with the help of Professor Stoker and was solely responsible for the statistical planning of the remaining 4 surveys; and Dr. M. L. Neser, who drew all the blood specimens (about 15 ml. each from a total of nearly 2,500 children of 6 - 15 years).

We wish also to acknowledge the invaluable assistance of Mrs. A. C. de Kock in the preparation of the literature survey.

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