Changes in Vitamin A and C Levels in Black Mine-Workers

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SUMMARY

An investigation of the vitamin A and C status of Black mine-workers was carried out at the request of the Anglo American Corporation of South Africa Ltd. It was found that, although there was no evidence of widespread vitamin A deficiency, there was sufficient evidence of subclinical deficiency to warrant further investigation. In spite of apparently adequate vitamin C intakes, a relatively high incidence of subclinical vitamin C deficiency was found.

It is strongly recommended that further studies be undertaken to establish what are the minimum levels of vitamin A and C required daily to reduce effectively the incidence of subclinical deficiency.

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In a world-wide survey of the incidence of xerophthalmia carried out by the World Health Organisation (WHO) (1962 - 63), the data collected by Escapini in Latin America, by Oomen in Asia, and by McLaren[1] in Africa, showed that xerophthalmia, a disease caused by severe vitamin A deficiency, remained the most important cause of blindness in the young child.

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 where there was no 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 number of premature deaths, thus reducing the numbers of xerophthalmia victims who survive to enter institutions.

The extent and character of the pathological changes produced by vitamin A deficiency 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:

1. Defective adaptation to the dark, due to a deficiency of the aldehyde form which is required for the production of rhodopsin.

2. Keratinisation of the mucous membranes in various parts of the body.

3. Defective skeletal architecture, due to a vitamin A deficiency during growth.

Since Lind's discovery in 1753 that 20 - 30 ml lemon juice daily would prevent scurvy, the human requirements of vitamin C have been extensively investigated. The minimum quantity of vitamin C that will prevent the development of scurvy has been established with reasonable certainty, but the desirable intake for the maintenance of optimum health remains unknown, and is the subject of considerable controversy. Infantile scurvy has been shown to occur only if the intake of vitamin C is less than 10 mg daily.[4-6] In a well-controlled study over a period of 12 months, the British Medical Research Council found that 10 mg vitamin C daily would prevent scurvy and also cure cases of scurvy which had developed on the basic diet.[7] This finding was confirmed by a study[8] on Canadian Air Force personnel, who did not develop scurvy on a daily intake of 8 mg vitamin C. Many authors[9-12] have studied the relationship between the intake of vitamin C and clinical symptoms, such as the condition of the gums.

It is well-known, however, that stress can cause an increase in vitamin C requirements, both in man[13-16] and in primates.[17] The role of infections in lowering serum vitamin C levels and increasing urinary excretion has been demonstrated by many experimental studies in guinea pigs.[18-19]

In children, otitis media, pneumonia, nephritis and other infections have long been recognised as precipitators of scurvy.[20] Malaria,[21] typhoid fever,[22] influenza,[23] measles,[24] tuberculosis[25] and vaccination against smallpox,[26] are among other specific infections where this effect has been reported.

In a study by Van Graan *et al.*[27] on Black mine-workers from a single mine, it was found that during the first 4 - 6 months of service, serum vitamin A levels decreased significantly, and that there was also an increase in the dark adaptation times. Furthermore, it is known that the morbidity rate of Black mine-workers is markedly higher during the first 3 months of service.[28] Clinical scurvy is

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occasionally seen in Black mine-workers, although the incidence is extremely low. (During 1972 6 cases of scurvy occurred in a population of more than 157 000 Black employees).[29]

At the request of the Medical Department of the Anglo American Corporation a study was undertaken to investigate the vitamin A and C status of Black mine-workers in the employ of member companies.

PATIENTS AND METHODS

As experimental subjects, Black mine-workers, who were working at 3 shafts of a gold-mining company at Orkney, were used. Two population groups were investigated, i.e. men from Botswana and so-called East-Coasters from Mozambique. Those from Botswana were from an arid area, while the East-Coasters were from a subtropical area. These populations were chosen in order to take possible differences in vitamin A liver reserves into account. During the 4 days of the study, all new arrivals from Botswana and Mozambique were investigated. The subjects under consideration were actually staying in 3 different hostels. The number of subjects from each hostel was known before the experiment. From some previous data on vitamin A levels, it was estimated that about 20 sample subjects should be included in each group to be studied, i.e. at each time point. These time points referred to the following periods of continuous service in the mine: new arrivals, 1 month (± 1 week), 3 months (± 1 week), 6 months (± 2 weeks), 12 months (± 1 month) and 24 months (± 1 month). In order to obtain at least 20 subjects in each group, it was decided to draw a sample of about 35 per group, where available. On this basis, a proportionate allocation was made to each hostel. This number of subjects was then selected sequentially at random from the qualifying inmates of each hostel.

In order to eliminate factors such as haemoconcentration, Black mine-workers were released from duty on the day of examination. Venous blood samples were taken, and the serum separated. Trichloracetic acid (TCA) extracts were made for vitamin C determination. TCA extracts and serum were frozen until assayed. Serum vitamin A and carotene concentrations were determined simultaneously, using the micromethod of Lowry *et al.*[9] Serum vitamin C was estimated, using the 2,4-dinitrophenylhydrazine method of Roe and Kuether.[30] Thiourea was used instead of Norit, as described by Schaffert and Kingsley.[31] Serum total proteins were estimated in a subsample (the first 10 subjects in each group), in order to eliminate the possibility that low serum vitamin A values might be due to a protein deficiency. Total serum protein was determined by the method of Weichselbaum,[32] and the protein fractions were separated by microzone electrophoresis on cellulose acetate, and scanned on the Spinco-Beckman analytrol.

It was originally intended to estimate the dietary intake of vitamins A and C by means of a small dietary survey. Since this proved to be impossible, it was decided to estimate dietary intake from total food purchases in relation to the number of people being fed. A survey was then done to establish whether certain food items were consistently being disregarded by the consumer in the choice of food at the self-service kitchens.

The three serum variables actually observed were: vitamin C (mg/100 ml), vitamin A (μg/100 ml) and carotene (μg/100 ml). Mean values and standard error of the means were computed at each time point. It was found that no sample subjects from Mozambique with 2 years of continuous service were available for study.

Firstly, it had to be established whether the Mozambique group differed significantly from the Botswana group of

TABLE I. MEAN VALUES, STANDARD ERRORS AND SAMPLE SIZES (given in this order)

Time of service (mo.)	Vitamin C		Vitamin A		Carotene	
	M	B	M	B	M	B
0	0,26	0,29	33	34	81	32
	0,032	0,027	1,8	1,6	8,3	2,1
	n = 41	n = 54	n = 37	n = 44	n = 37	n = 44
1	0,10	0,26	25	25	90	52
	0,015	0,03	1,7	1,4	6,6	3,6
	n = 27	n = 23	n = 26	n = 23	n = 26	n = 23
3	0,08	0,16	34	37	72	63
	0,011	0,014	2,1	2,2	4,3	5,4
	n = 27	n = 20	n = 27	n = 21	n = 27	n = 21
6	0,12	0,20	31	33	70	63
	0,010	0,024	2,2	2,1	3,7	4,0
	n = 23	n = 30	n = 25	n = 31	n = 25	n = 31
12	0,29	0,13	35	34	82	56
	0,026	0,012	1,1	2,3	8,1	3,7
	n = 29	n = 22	n = 29	n = 23	n = 29	n = 23
24		0,08		29		57
	—	0,020	—	1,7	—	4,7
		n = 24		n = 24		n = 24

M = Mozambique; B = Botswana.

subjects with regard to mean values and trend. Graphs prepared from Table I clearly demonstrate that an overall analysis is not possible, since the time trends are markedly different, especially for vitamin C levels. Thus, separate comparisons were made at each time point, by using a series of Student's t-tests.[33] In cases where the variances differed significantly, this was treated as a Behrens-Fisher[33] problem.

Secondly, possible significant differences in time of continuous service were established for the Mozambique and Botswana groups, each treated separately. The Scheffé multiple comparison test[34] was utilised in this case.

RESULTS

The means, standard error of the means, and sample sizes obtained in the study, are listed in Table I and illustrated in Figs 1 - 3.

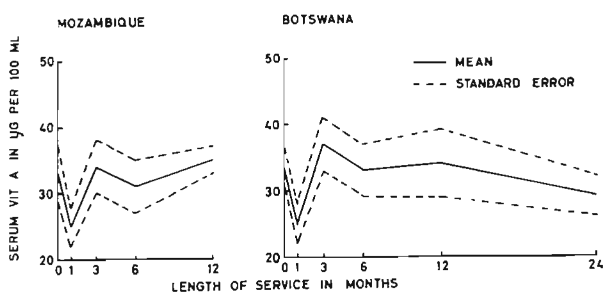

Fig. 1. Mean serum vitamin A values ± 2 × standard error.

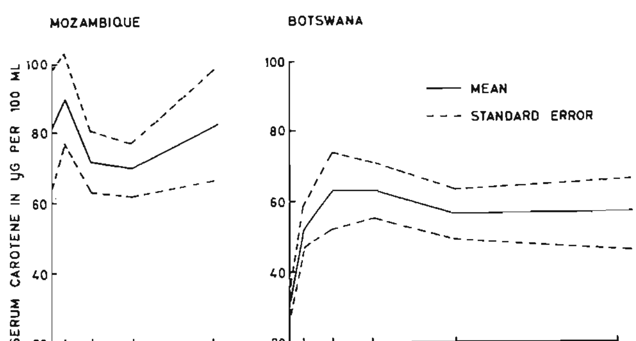

Fig. 2. Mean serum carotene values ± 2 × standard error.

From Table I it is obvious that there is very little difference in terms of vitamin A and C levels between subjects from Botswana and Mozambique when they arrive. As for vitamin A levels, there was little difference between the responses of the two groups. There was a marked decline in serum vitamin A levels in both populations after 1 month of service, with recovery at 3 months to levels slightly above those obtained on arrival.

The results of comparing the mean values of the Mozambique and Botswana groups at different time points are presented in Table II. Entries in Table II are exceedance probabilities of the Student's t-statistic. Small probabilities indicate that the computed value of the t-statistic is a rare event, which means that the hypothesis of equal means is rejected in those cases.

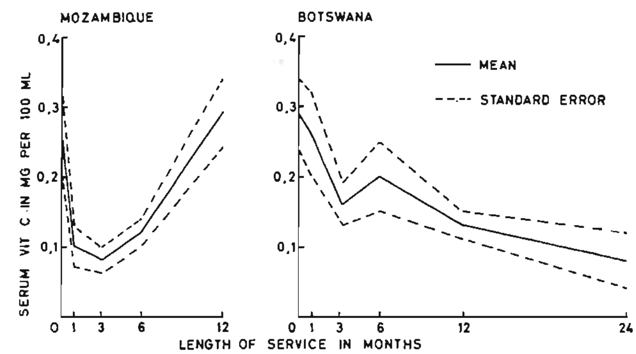

Fig. 3. Mean serum vitamin C values ± 2 × standard error.

TABLE II. COMPARISON OF MEAN VALUES OF THE MOZAMBIQUE GROUP WITH THE BOTSWANA GROUP

Time of service (mo.)	Vitamin C	Vitamin A	Carotene
0	0,494	0,539	0,000†
1	0,000*	0,853	0,000†
3	0,000*	0,286	0,220
6	0,004*	0,504	0,177
12	0,000†	0,770	0,006†

* Differences in group means significant at the indicated probability level. Group means for Botswana data exceed those of the Mozambique data.
† Differences in group means significant at the indicated level. Group means for Mozambique data exceed those of the Botswana data.
The entries in the Table are exceedance probabilities of the Student's t-test.

Considering the vitamin A levels in conjunction with the results of the tests listed in Table II, we conclude that no significant differences exist between the average responses of the Mozambique and Botswana groups.

It is generally accepted that carotene has no active physiological function in the body, and can be neither used nor stored, unless converted to vitamin A. It is thus obvious that low serum carotene levels are not necessarily indicative of vitamin A deficiency. As could be expected, considering the respective climates, carotene levels in the subjects from Mozambique were substantially higher on arrival than those obtained from subjects from Botswana. Once on the mine diet, there was a marked increase in carotene values found in the groups from Botswana, whereas there was a decrease in corresponding groups from Mozambique.

In terms of vitamin C, the response of the two groups was markedly different. The Mozambique group showed a very pronounced decline in serum vitamin C levels, reaching a minimum in the group with 3 months' service.

At 6 months, a slight improvement in serum vitamin C levels was found, and values similar to those on arrival were obtained after 12 months of service. For the Botswana groups, however, the decrease in vitamin C levels over the first 3 months was not as marked as it was for the Mozambique group. Once again, there was a slight improvement in the levels found in the group with 6 months' service as compared with those with 3 months' service. The mean vitamin C level for the Botswana groups with service longer than 6 months showed a marked decline, reaching a minimum in the group with 24 months' service.

Upon arrival there were no significant differences between the groups from Mozambique and Botswana. However, at all stages of service thereafter, highly significant differences were found.

A further purpose of our analysis was to study the trend of mean values with time for the Mozambique and Botswana groups taken separately. The results of Scheffé multiple comparisons are contained in Table III.

TABLE III. PAIRWISE COMPARISONS OF GROUP MEANS

	Vitamin C	Vitamin A	Carotene
Mozambique	3;1;6;0;12	1;6;0;3;12	6;3;0;12;1
Botswana	24;12;3;6;1;0	1;24;6;0;12;3	0;1;12;24;6;3

The first entry in each cell denotes the time of continuous service at which the smallest sample mean was observed etc., in ascending rank order. If two means do not differ significantly from one another at an over-all 5% level of significance, they are underlined by a common line. For example, in the case of carotene for the Mozambique group, not a single pair of significantly different means was found. For vitamin A, the 1-, 6-, 0- and 3-month groups do not differ significantly from one another, and the same applies to the means for the 6-, 0-, 3- and 12-month groups. These results emphasise the fact that the growth patterns for the three variables are rather different for the two groups being studied.

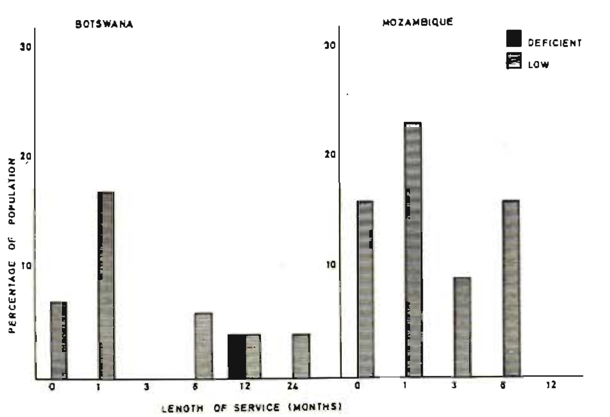

Fig. 4. Incidence of 'deficient' and 'low' serum vitamin A values.

Individual results were classified according to the scale of interpretaion proposed by the Interdepartmental Committee on Nutrition for National Defense (ICNND).[35] Figs 4 and 5 illustrate the percentage incidence of subjects of serum values within the 'deficient' and 'low' ranges.

According to the ICNND, the presence of 5% or more subjects with serum vitamin A values below 10 μg/100 ml (deficient range), or 15% with values blow 20 μg/100 ml (10 - 19 μg/100 ml = low range), is indicative of a vitamin A deficiency in the population as a whole.

From Fig. 4 it can be seen that, as a whole, the two populations cannot be considered to be deficient in vitamin A, according to ICNND standards. There are, however, groups within these populations where the percentage incidence was higher than 15%. Total serum albumin contents were estimated for the first 10 subjects in each subgroup from both Mozambique and Botswana. This subset of the sample will remain representative of the population, in view of the sequential sampling technique used.

TABLE IV. MEANS AND STANDARD DEVIATION FOR SERUM ALBUMIN

Length of service (mo.)	Mozambique	Botswana
0	4,33 ± 0,14 g/100 ml	4,44 ± 0,20 g/100 ml
1	4,46 ± 0,36 g/100 ml	4,52 ± 0,33 g/100 ml
3	4,42 ± 0,33 g/100 ml	4,47 ± 0,34 g/100 ml
6	4,08 ± 0,27 g/100 ml	4,41 ± 0,21 g/100 ml
12	4,26 ± 0,27 g/100 ml	4,40 ± 0,21 g/100 ml
24		4,69 ± 0,36 g/100 ml
Total group mean and SD	**4,30 ± 0,29 g/100 ml**	**4,50 ± 0,28 g/100 ml**

From Table IV it is obvious, however, that there is no indication of low serum albumin values which could be considered indicative of protein deficiency in any of the groups. Protein deficiency can thus safely be disregarded as a cause of low serum vitamin A values.

According to Van Graan *et al.*[27] it could be expected that the time taken by individuals to adapt to the dark in the low vitamin A range of nutrition would be increased. Under mining conditions, where the level of illumination is often very low, any inhibition of dark adaptation capacity in individuals could thus be of major importance. From Fig. 4 it is obvious that the incidence of low serum vitamin A values is sufficiently high to warrant further investigation into possible remedial measures to eliminate or effectively reduce such low values in the mining population.

From Fig. 5 it is evident that vitamin C deficiency constitutes a problem of considerable magnitude. For the Mozambique population, a maximum incidence of 'deficient' and 'low' values was found in the group which had been at the mine for 3 months, whereas the maximum incidence in the Botswana group was found after 24 months of service.

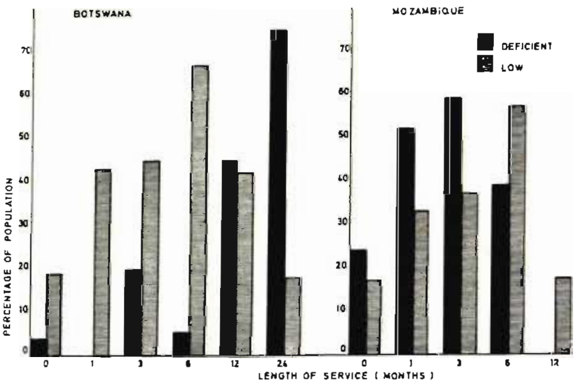

Fig. 5. Incidence of 'deficient' and 'low' serum vitamin C values.

DISCUSSION

With regard to vitamin C nutrition status at varying lengths of service, there is no obvious explanation for the differences found in the two populations involved (Table I and Fig. 5). If it is accepted, however, that psychological stress could reduce serum vitamin C levels and increase vitamin C requirements in man, it is possible that such stress could explain these findings. It is possible, therefore, that the subjects from Mozambique fully adapt to the initial stress undergone when starting to work on a mine, and that in subjects from Botswana secondary stress factors become operative after approximately 6 months of service. There did not seem to be any adaptation to the secondary stress, since minimum serum vitamin C was obtained at 24 months.

CONCLUSION

It was initially intended to carry out a dietetic evaluation of vitamin A and C intakes. Under conditions prevailing at the feeding centres of the mine (the chief problem being the rate at which individuals passed through service kitchens), it was impossible to make an accurate assessment of dietary intakes. It was therefore decided to base the dietary estimates on total food purchases of a kitchen in relation to the number of mine-workers fed. Simultaneously, a small survey was made to establish whether certain food items were selectively disregarded by the mine-workers in their choice of food at the self-service kitchens.

No clear-cut evidence of bias against any food items was found for the population being fed from the kitchen. At the rate of feeding (i.e. 1 200/hour), it was impossible to differentiate between the subjects from Mozambique and those from Botswana. Vegetables of various kinds were provided at a rate of 340 g/head/day. It was estimated that after preparation the vegetable intake was 220 g/head/day. *Magou*, fortified with vitamin C, was invariably taken by everyone. The amount of vitamin C added should provide approximately 35 mg/head/day. In addition to the vegetable intake, tomato products are consumed at a rate of approximately 30 g/head/day. A conservative deduction can thus be made that the daily vitamin C intake is not less than 50 mg/day. This estimated intake compares extremely well with the recommended daily requirements.[36]

It is thus obvious that the low serum values found cannot be explained by any deficiency in dietary intake. Under the stress of mining conditions, relatively widespread subclinical vitamin C deficiency may therefore occur, in spite of what, under other conditions, would be an adequate dietary intake. Further studies will be required to establish what would be the minimum level of vitamin C intake that would be effective in maintaining adequate serum levels in these circumstances.

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