Studies on the serum total carotenoids, vitamin A and serum colour in Nigerian soldiers

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In Western Nigeria samples of serum or plasma are often bright yellow and yet have normal levels of bilirubin. In a pilot experiment in 1959 we found that unusually high levels of total carotenoids were present in several samples of yellow serums. Edozien (1960) has since reported values for serum carotene in Ibadan ranging from 150 to 900 μg/100 ml in normal individuals, compared with the figure of 200 μg/100 ml accepted as the upper limit of normal in persons living in the United Kingdom (Moore, 1957; Varley, 1958). Edozien suggested an association with the dietary intake of palm oil and peppers. More recently Dagadu & Gillman (1963) have reported values above 500 μg/100 ml in 80% of individuals in a study in Accra, Ghana.

In the investigation here reported the observations of the pilot experiment were expanded by a study of the relation between the total carotenoid levels of the serums and their yellowness as measured by the icteric index. The values for serum total carotenoids in healthy Nigerian soldiers obtained during this study form a useful addition to Edozien's (1960) figures, as they refer to a different city (Lagos) and have been obtained for a group of soldiers living on a diet rather better than the regional average. At the same time the vitamin A levels were studied, and in a few individuals it was also possible to measure α-tocopherol levels, for which no data are as yet available from this city. A survey of the diet was made to discover what sources of carotenoids were present and to assess its adequacy. The carotene content of a local sample of palm oil was also measured.

Our findings confirmed that the total carotenoid levels in serum were above European levels, often by a considerable amount, and correlated well with the yellowness of the serums, provided that the bilirubin was not abnormally elevated. Vitamin A levels were found to be towards the upper limits of the accepted range of normal values, and α-tocopherol was within or slightly below the normal range.

EXPERIMENTAL

Analytical methods

The yellowness of the serums was estimated by direct visual comparison with the icteric index standards prepared as directed by Varley (1958). No attempt was made

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to use a tintometer or spectrophotometer for this estimation, since it was the visual impression of yellowness that formed the basis of this investigation.

Bilirubin was measured by the method of Malloy & Evelyn (1937). Both absolute bilirubin standards (Hoffman-La Roche Ltd) and methyl red standards were used.

Total carotenoids were measured by the method of Varley (1958), the extinction of the extract being read at 450 μm (not 440 μm as directed by Varley). The readings were calibrated against those for β-carotene (Roche Products Ltd) freshly prepared from a sealed ampoule.

Vitamin A was measured by the antimony trichloride method recommended for blood samples by Embree, Ames, Lehman & Harris (1957) with the two exceptions of detail that (*a*) the Unicam SP 600 was used in place of the Evelyn colorimeter, with an adapter to permit mixing of reagents whilst in the light beam, and (*b*) the carotenoids were measured at 450 μm, not 440 μm as directed. Standard curves were prepared for vitamin A, ampoules of palmitate of activity 1 000 000 i.u./ml supplied by British Drug Houses Ltd being used, or with samples from bulk supplies of the same activity from Roche Products Ltd. Standard curves for carotenoids were prepared with the same standards as were used for total carotenoid determination.

α-Tocopherol was measured by Mr J. G. Lines, Department of Medical Biochemistry and Pharmacology, University of Birmingham, who used a method shortly to be published of elution from lipid paper chromatograms.

The Unicam SP 600 spectrophotometer was used for all measurements in the visible range, and the SP 500 for the u.v. determinations of α-tocopherol.

Pilot experiment

Four serums selected from the routine laboratory material at the Military Hospital were dispatched by air mail to the United Kingdom. They travelled at room and aircraft temperature, and suffered a 3 weeks' delay in transit owing to a misunderstanding over Customs clearance, during which they were stored at $+4^{\circ}$.

Main experiment

Twenty representative subjects (volunteers) were selected from a Royal Nigerian Army unit stationed in Lagos. Their ages ranged from 23 to 42 years (mean 28·7 years). The length of stay in Lagos varied from 1 to 17 years (mean 5·5 years), and the duration of army service was from 2 to 20 years (mean 8·1 years). The majority gave a history of past attacks of fever, probably due to *Plasmodium falciparum* (Welch) which is a frequent cause of illness in the coastal belt of Nigeria. All had taken Daraprim (pyrimethamine; Burroughs Wellcome and Co., London) as a malarial prophylactic under supervision for at least 1 year. Five subjects (nos. 2, 6, 11, 15 and 16) gave a history of jaundice within the last 5 years. The subjects were examined medically, and all were found to be of good physique, without any evidence of present illness or of recent vitamin deficiency.

All were taking a fairly constant diet of the local variety, except for one (no. 19) who ate European dishes on alternate days. The diet was investigated by means of a set questionnaire concerning all known local items of food, and by visiting the quarters of the individuals concerned (and others) to observe the meals taken and, wherever possible, to weigh the quantities involved.

The subjects were all bled on the same day, and the samples were allowed to clot in the dark. The serum was separated without exposure to sunlight and the samples were dispatched by air freight the same day in a vacuum container which was initially ice cold (0°). They were received in Birmingham 3 days later, when the total carotenoids and icteric index were determined on the day of receipt. After storage at +4° in the dark, the bilirubin was determined the next day, and most of the vitamin A levels were determined during the next 5 days. A few samples could not be analysed until 2 weeks had elapsed from the time of taking. α-Tocopherol was determined after 1 week's storage.

Additional experiment

Serums originally dispatched to the United Kingdom for liver function tests were used to provide additional values. They travelled at room and aircraft temperature without the precautions stated above, and had been stored for 1 week at +4° in the dark before being measured. They were taken from patients recovering from gastroenteritis, schistosomiasis, or pyrexia of undetermined origin.

RESULTS

Pilot experiment

Despite the delay encountered in obtaining the samples for analysis, they were still unusually yellow on arrival. The bilirubin level was less than 0·5 mg/100 ml in all four, and in the two yellowest samples the total carotenoid levels were 550 and 245 μg/100 ml. When the serums had stood for 1 week at room temperature in daylight the yellowness had faded and the quantity of total carotenoids had fallen to 45 and 55 μg/100 ml.

Main and additional experiments

The results for the twenty subjects in the main experiment are set out in Table 1 and those for the ward patients in the additional experiment in Table 2. The correlation between the yellowness, as judged visually, and the serum total carotenoids for both sets of subjects is shown in Fig. 1. It is clear that the variability of the level of bilirubin disturbed this correlation, and the individuals with bilirubin levels above 0·5 mg/100 ml are indicated on the figure.

The icteric index of fresh serums (main experiment) ranged from 5 to 22 units compared with a normal European upper limit of 6 units (Varley, 1958). In stored serums (additional experiment) it ranged from 2 to 17 units, excluding the patient with abnormal bilirubinaemia (no. 42). Serum total carotenoids ranged from 158 to 631 μg/100 ml (mean 300 μg/100 ml) for fresh serums, and from 132 to 532 μg/100 ml in stored serum. Of the forty-two individuals tested, five had levels over 500 μg/100 ml and if white-skinned would undoubtedly have presented the features of carotinaemia (Cohen, 1958). Bilirubin values below 0·5 mg/100 ml were not recorded, as the method used in this laboratory cannot be regarded as wholly reliable below this figure. They

Table 1. *Main experiment. Results of determinations made on serums from twenty healthy Nigerian soldiers*

Soldier no.	Icteric index (units)	Serum total carotenoids ($\mu g/100$ ml)	Bilirubin (mg/100 ml)	Vitamin A (i.u./100 ml)	α-Tocopherol (μg/ml)
1	12	295	< 0·5	289	—
2	8	191	< 0·5	266	—
3	14	270	< 0·5	175	—
4	10	215	< 0·5	162	6·1
5	20	598	0·7	202	—
6	7	166	1·1	268	—
7	8	233	0·9	288	—
8	20	348	0·5	230	—
9	14	631	< 0·5	197	—
10	6	262	< 0·5	195	4·4
11	10	179	0·9	176	—
12	18	374	0·7	146*	—
13	22	543	< 0·5	—	—
14	10	333	< 0·5	130*	—
15	5	158	< 0·5	—	—
16	16	192	0·9	—	—
17	22	167	0·7	105*	—
18	16	290	< 0·5	—	4·9
19	18	283	< 0·5	127*	9·5
20	14	270	0·8	186*	—

* Determinations not performed until the serums had been stored for 2 weeks at +4°.

Table 2. *Additional experiment. Results of determinations made on serums stored for 1 week at +4°, obtained from Nigerian soldiers recovering from the conditions shown in the last column*

Soldier no.	Icteric index (units)	Serum total carotenoids ($\mu g/100$ ml)	Bilirubin (mg/100 ml)	Vitamin A (i.u./100 ml)	Clinical diagnosis
21	2	132	< 0·5	240	Treated schistosomiasis
22	5	264	< 0·5	270	Amoebiasis and gastroenteritis
23	10	344	< 0·5	130	Gastroenteritis
24	11	346	< 0·5	130	Gastroenteritis
25	7	188	< 0·5	205	Gastroenteritis and malaria
26	4	220	< 0·5	167	Gastroenteritis
27	6	406	< 0·5	195	Gastroenteritis
28	5	200	< 0·5	—	Schistosomiasis (active)
29	15	512	< 0·5	158	Gastroenteritis and malaria
30	12	250	< 0·5	178	Treated schistosomiasis
31	6	160	< 0·5	218	Gastroenteritis
32	10	252	< 0·5	—	Treated schistosomiasis
33	6	250	< 0·5	—	Schistosomiasis (active)
34	11	355	< 0·5	—	Schistosomiasis (active)
35	8	270	< 0·5	—	Schistosomiasis (active)
36	8	170	0·8	—	Schistosomiasis (active)
37	6	235	< 0·5	—	Amoebiasis and gastroenteritis
38	17	355	< 0·5	—	Respiratory infection
39	13	532	< 0·5	—	Pyrexia of undetermined origin
40	4	195	< 0·5	—	Schistosomiasis and pyrexia of undetermined origin
41	7	292	< 0·5	—	Gastroenteritis and amoebiasis
42	20	295	1·5	—	Gastroenteritis

ranged from less than 0·5 to 1·1 mg/100 ml in fresh serums and were rather lower in stored serums. Edozien (1958, 1960) quotes a normal range of 0·25–1·0 mg/100 ml, and no. 42 was the only subject considered to have an abnormal value.

The correlation coefficient between the serum total carotenoids and the icteric index (Fig. 1) was +0·51 with a standard error of 0·16. The regression equation was:

$$\text{total carotenoids } (\mu g/100 \text{ ml}) = 174 + 11{\cdot}4 \times \text{icteric index (units)}.$$

Vitamin A levels, all determined at 1 week after venepuncture, ranged from 130 to 289 i.u./100 ml (mean 193 i.u./100 ml), compared with a range for Europe and the USA of 60–280 i.u./100 ml with a mean of 131 i.u./100 ml (Moore, 1957). The levels

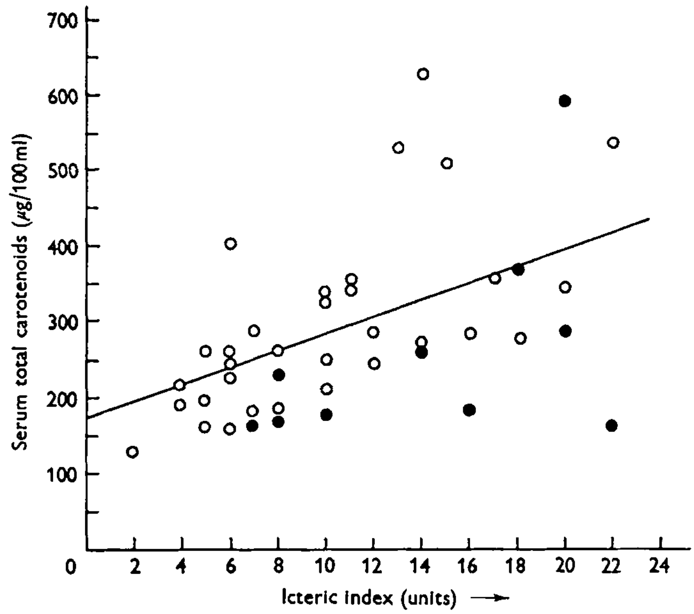

Fig. 1. Correlation between serum total carotenoids and icteric index in the forty-two subjects in the main and additional experiments. ○, subjects with serum bilirubin below or equal to 0·5 mg/100 ml; ●, subjects with bilirubin above 0·5 mg/100 ml. The line is the calculated regression line, the equation being: total carotenoids (μg/100 ml) = 174+11·4 × icteric index (units).

were thus within the wide normal limits though often higher than those quoted for the United Kingdom (Varley, 1958), and they tended to lie well in the upper half of the distribution nomogram (Moore, 1957, p. 361).

The α-tocopherol levels were within or below the normal European range of 3·5–9·0 μg/ml, the lower levels probably being attributable to the delay in measurement.

Palm oil

A representative sample of palm oil was obtained from the market most used by individuals in this study. It was orange-red, melting between 22° and 28°, completely soluble in light petroleum and heptane, and gave a value for total carotenoid content of 740 μg/g at 450 μm. This was a typical example of the pericarp oil of the oil-palm nut (Oliver, 1958; Moore, 1957).

Diet

The survey of the subjects' diet showed that it was of fairly high calorie content, and contained adequate amounts of protein and fat. The daily intake was usually between 3000 and 3500 kcal. The recorded weights of the subjects ranged from 58 to 81 kg with a mean of 64·7 kg. Table 3 shows the usual daily consumption of the most important carotenoid-containing foods in the diet.

Table 3. *Amounts and carotene content (McCance & Widdowson,* 1960*; Moore,* 1957) *of the major sources of carotenoids in the diet of the Nigerian soldiers*

Item	Typical amount eaten daily (g)	Carotenoids supplied daily (i.u.)
Palm oil	100–200	60000–120000
Yam	450–600	25000–35000
Beans	100–150	500–1000
Tomatoes	100–120	1200–1400
Oranges	200–300	500–700

DISCUSSION

It has been confirmed that the range of serum total carotenoid values in healthy and convalescent Nigerians in Lagos is above that usually accepted as normal in the United Kingdom. These values were found to be significantly correlated with the yellowness of serum samples. A survey of the diet showed that the intake of palm oil was considerable and would contribute daily 60–120 mg of carotenoids. This is a large amount, equivalent to the ingestion of 0·5–2·0 kg of raw carrots daily (Moore, 1957; McCance & Widdowson, 1960). In clinical cases of carotinaemia the reported intakes were between 0·5 and 1·0 kg of carrots per day (McConaghey, 1952; Auckland 1952). In experimental feeding of β-carotene to human volunteers (Urbach, Hickman & Harris, 1952) it was found that 62 mg daily were able to raise the serum carotene to 450 μg/100 ml, and that it could be maintained at this level by as little as 28 mg daily. It is therefore evident that the quantity taken in palm oil alone was sufficient to explain the levels observed.

The other sources of carotene in the diet that might make a significant contribution were yam, for which James & Hollinger (1954) have reported a fairly low absorption of the available carotene, tomatoes, and peppers. The absorption of carotene is greatly influenced by the presence of fat in the diet (Roels, Trout & Dujacquier, 1958), and the administration of the carotene dissolved in a lipid, as it is in palm oil, has a more dramatic effect upon blood levels than when it is an ingredient of vegetables. Hence it seems probable that the palm oil is the most important factor in the observed high carotenoid levels in these subjects.

It is also noteworthy that there should have been five individuals with total serum carotenoid levels above 500 μg/100 ml, as these levels in white-skinned people would undoubtedly cause visible pigmentation with the characteristic distribution of carotinaemia (Cohen, 1958; McConaghey, 1952). This condition, usually regarded as rare, is now seen to be common in West Africa, where, if our figures, Edozien's (1960) and those of Dagadu & Gillman (1963) are typical, there must be many hundreds of thousands of cases. Our findings also serve to explain another feature of medical study in these areas, which is the unusual brown colour of adult human fat when seen at operation and necropsy. It has been reported (Peirce, 1954) that carotene has the effect of so colouring fat, and that carotene thus deposited is relatively inaccessible to the body for metabolic purposes.

As it was expected that carotenoid levels would be found to be high in this study, the measurement of vitamin A levels was a natural corollary. The carotenoids are precursors for vitamin A synthesis, and Cohen (1958) has consistently noted elevation of vitamin A levels in clinical carotinaemia. By contrast, Urbach *et al.* (1952) were unable to show any rise in vitamin A levels when they caused the serum carotene to rise to 450 μg/100 ml in volunteers, but they only conducted their observations for a limited period, and also recorded that large doses of vitamin A had little effect on the serum levels in their subjects. These findings suggest that storage sites in the body, probably mainly the liver, need to be filled by excess vitamin A before significant changes in level occur in the blood. In this connexion it may be noted that Dagadu & Gillman (1963) have found increased vitamin A stores in human livers obtained at necropsy in Accra. Our results show that many individuals had appreciably elevated serum levels of vitamin A, but that this elevation was not similar in degree to that of the serum total carotenoids, nor could any correlation with carotenoid level be demonstrated. The observations are in agreement with those of Dagadu & Gillman (1963) who also found elevation of vitamin A levels in the serum of some of their subjects, and lack of correlation between vitamin A and carotene levels. However, our findings are at variance with those of Edozien (1960), who found at Ibadan levels of vitamin A ranging from 11 to 40 μg/100 ml (i.e. 36–130 i.u./100 ml); this difference may be due to dietary differences between the cities of Ibadan and Lagos, or to the fact that our sample was of people having an unusually good diet by local standards.

SUMMARY

1. Serums in Western Nigeria are unusually yellow and high serum total carotenoid values may occur.

2. After a pilot survey, serum total carotenoids, icteric index and bilirubin were determined in twenty healthy Nigerian soldiers, and in twenty-two convalescent soldiers.

3. The carotene values were found to be high and to correlate significantly with the yellowness of the serum, especially when bilirubin content was taken into account.

4. Vitamin A levels were found to be moderately elevated.

5. The diet was investigated, and the intake of carotenoids in palm oil alone was found to be adequate to explain the high levels of serum carotene found.

6. A small number of α-tocopherol estimations were performed, and levels were low or normal.

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