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VITAMIN A, TOTAL CAROTENOIDS, AND THYMOL TURBIDITY LEVELS IN PLASMA

TEST IN NORMAL SUBJECTS RESIDING IN THE MIDLANDS DURING 1947

BY

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In the course of a previous investigation into the cause of nystagmus in coal-miners we estimated the levels of vitamin A and total carotenoids in the plasma (Campbell and Tonks, 1948). Thymol turbidity tests were performed at the same time on a number of cases in order to exclude the possibility of liver dysfunction as a cause of fluctuation in these constituents. Since the diet of the majority of people had been rather drastically altered by war and post-war conditions in Great Britain, we were obliged to establish "normal" figures for comparison. It was thought that the values obtained might be of practical interest to other investigators engaged on similar problems.

Methods

Estimation of Vitamin A and Total Carotenoids in Plasma

Clausen's application of the Carr–Price colorimeter method was the means used in this procedure. The colour was measured in a Hilger absorptiometer, using filters specially designed for these estimations. The results were read off from standard curves prepared as suggested by Yudkin (1941).

The following reagents were used: (1) ethyl alcohol (redistilled); (2) petroleum ether (boiling point 40–60° C.); (3) chloroform—alcohol-free and dried over anhydrous sodium sulphate; (4) antimony trichloride solution 30% (w/v) in dry chloroform; this is a saturated solution freshly prepared and filtered, and is cleared of any turbidity by the addition of one drop of acetic anhydride.

Preparation of Standard Curves.—Carotenoids:—For this purpose pure β-carotene was obtained from the National Institute for Medical Research. A weighed amount (about 0.25 mg.) was dissolved in 100 ml. of petroleum ether (boiling point 40–60° C.). Aliquots of this solution were made up to 5 ml. with petroleum ether, and readings were taken in the absorptiometer to determine the standard curve for β-carotene, using the special filter combination provided by Hilger for carotenoids. *Vitamin A:*—Capsules of vitamin A alcohol of high purity were very kindly given to us by Roche Products, Ltd., to whom we offer our thanks. The contents of one capsule were weighed and made up to a known volume with chloroform. Under our conditions 1 ml. of this solution contained 27.25 i.u. Each aliquot taken was made up to 0.5 ml. with chloroform, and then 5 ml. of antimony trichloride solution was added. The resulting colour was read off on the absorptiometer within 10 seconds of the addition of the reagent, using the special combination of filters designed by Hilger for the estimation of vitamin A.

Correction Curve for the Blue Colour Produced by β-carotene and Antimony Trichloride.—A solution of a weighed quantity of β-carotene (1.2 mg.) was made up to 100 ml. with chloroform. Aliquots were made up to 0.5 ml., and 5 ml. of antimony trichloride solution was added. The special filter for vitamin A was used in the absorptiometer, and readings were taken within 10 seconds of mixing the reagents.

From these readings graphs were constructed so that results could be ascertained with accuracy and speed.

Two millilitres of plasma was placed in a stoppered centrifuge tube, 2 ml. of ethyl alcohol was added slowly, and the contents thoroughly mixed. To this mixture was added 4 ml. of petroleum ether. The stopper was inserted and the tube shaken at frequent intervals for half an hour. The mixture was then allowed to settle into its two layers or was centrifuged. An aliquot of the top layer (usually 3.6 ml.) was then transferred to an absorption cell, made up to 5 ml. with petroleum ether, and a reading taken using the carotenoid filter. The equivalent value in terms of β-carotene was then obtained from the standard curves and calculated for 100 ml. of plasma.

The petroleum ether solution in the cell was evaporated to dryness by placing the cell in a bath of warm water (40–60° C.). A gentle current of dry air or nitrogen was used to assist evaporation. The residue was dissolved in 0.5 ml. of dry chloroform. With the use of the special vitamin-A filter the cell was placed in the rack of the absorptiometer and 5 ml. of antimony trichloride solution added. The reading was taken within 10 seconds of the addition of the reagent.

The amount of vitamin A present in the cell was calculated by reading the value from the standard curve for vitamin A and subtracting the amount of blue colour due to the reaction of the antimony trichloride with the estimated amount of carotenoids present. From this value the amount in 100 ml. of plasma was calculated.

Thymol Turbidity Test

This test was originally described by Maclagan (1944), but in order to get more precise comparable values the modification of Ley, Lewis, and Davidson (1946) was used, in which a barium sulphate suspension was employed as a standard and the turbidity was measured in the Hilger absorptiometer. The results given in this paper, therefore, are expressed as being equivalent to the corresponding amount in millilitres of barium sulphate suspension.

A slight modification was introduced—namely, the performance of a blank test with 0.1 ml. of plasma and 6 ml. of 0.9% sodium chloride. The reading of this blank test on the standard barium sulphate suspension curve was subtracted from the value obtained in the usual way in the case of lipaemic or slightly haemolysed plasma.

Results

Vitamin A and Total Carotenoids

Our series consisted of 133 normal adults (116 men and 17 women) varying in age from 18 to 73 years. They included housewives, office workers, factory hands of all types, and labourers, and came from all walks of life. They were tested only when free from infection, as Brenner and Roberts (1943) found that feverish colds reduced the blood level of vitamin A by one-half. All specimens of blood were collected at approximately three hours after

a meal, but no attempt was made to obtain fasting specimens, since Kimble (1939) found that ordinary meals do not cause a measurable increase in either vitamin A or carotene within two to six hours.

The figures in our 133 subjects were:

Total carotenoids (μg./100 ml.)	20–199	Mean 80
Vitamin A (i.u./100 ml.)	69–158	„ 108

These figures are strikingly different from those of Haig and Patek (1942) in America, based on 74 measurements on 44 normal people between the ages of 20 and 45:

Total carotenoids (μg./100 ml.)	69–240	Mean 144
Vitamin A (i.u./100 ml.)	109–309	„ 198

It is to be noted that all these subjects were on highly nutritious diets which were estimated to provide at least 13,000 i.u. of vitamin A daily.

Yudkin (1941) in England, in a series of 23 normal adults varying from 18 to 30 years of age, found:

Total carotenoids (μg./100 ml.)	50–240	Mean 120
Vitamin A (i.u./100 ml.)	72–157	„ 113

while Moore and Leitner, during the course of an investigation of the vitamin A requirement of human adults, published in the Medical Research Council Special Report No. 264 (1949), collected a series of 195 cases, comprising 112 subjects with illnesses not expected to affect the vitamin A value, and 83 normal subjects. They give the following figures as their mean values:

	All Cases	Normals
Total carotenoids (μg./100 ml.)	92	100
Vitamin A (i.u./100 ml.)	121	128

Their series was collected from residents in the London area in 1946, while ours, collected during 1947 in the Midlands, are lower than these, both in carotenoids and in vitamin A levels.

The long-continued low intake of fat due to rationing restrictions accounts for the fact that our standards for the average blood level of carotenoids and vitamin A in this country are lower than those considered normal by American standards. This must be borne in mind when assessing the nutritional state of persons suffering from disease.

Variation with Age.—Taking our cases in approximately 10-year age groups, the total carotenoids and vitamin A figures show a slight variation with age (Table I).

Influence of Season.—There was a slight fluctuation in the vitamin A values when grouped in two-monthly periods throughout the year, while the carotenoids showed a steady rise from March/April to November/December (Table III).

TABLE III.—*Seasonal Variation in Total Carotenoid and Vitamin A Content of Blood Plasma*

	Jan. Feb.	Mar. Apr.	May June	July Aug.	Sept. Oct.	Nov. Dec.
Total carotenoids (μg./100 ml.)	82	65	69	75	82	97
Vitamin A (i.u./100 ml.)	110	94	108	113	108	112

Influence of Occupation.—The cases investigated have been grouped into four categories to see if any difference in carotenoid and vitamin A blood levels resulted from hard physical exercise and consequent increased metabolism (Table IV). The "heavy" category of occupation in this series included all those people who do hard manual labour, with the exception of coal-miners. An additional group of 133 miners (divided into "surface" and "underground" workers) shows figures comparable with those for other heavy industries, the levels being particularly low in miners working underground (Table V).

TABLE IV.—*Influence of Occupation on Total Carotenoid and Vitamin A Content of Blood Plasma*

	Occupation			
	Heavy	Medium	Light	Sedentary
Total carotenoids (μg./100 ml.)	76 (37 cases)	79 (34 cases)	88 (32 cases)	81 (30 cases)
Vitamin A (i.u./100 ml.)	102	109	111	113

TABLE V.—*Variation of Total Carotenoid and Vitamin A Content of Blood Plasma in Coal-miners*

	Miners, Surface	Miners, Underground
Total carotenoids (μg./100 ml.)	72 (53 cases)	67 (80 cases)
Vitamin A (i.u./100 ml.)	102	97

Thymol Turbidity Tests

Thymol turbidity tests were performed on 110 normal adults, giving values varying from 0.2 to 1.85 ml. of barium sulphate suspension with a mean value of 0.59. Ley, Lewis, and Davidson (1946), in a series of 105 healthy young men, found a range of 0.16 to 2.19 ml. with a mean value of 0.63. In our series 84% of the cases had a value of less than 0.9 ml. and 93% a value of less than 1.06 ml. of barium sulphate suspension, while less than 3% had values exceeding 1.5 ml.

It is suggested that the introduction of the blank reaction into this estimation has the effect of reducing the upper limit of the normal range, and that the true range should be 0 to 1.5 ml. of barium sulphate suspension.

TABLE I.—*Age Variation in Total Carotenoid and Vitamin A Content of Blood Plasma*

	18–29 Years		30–39 Years		40–49 Years		50–59 Years		60–73 Years	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Total carotenoids (μg./100 ml.)	20–118	70 (26)	42–199	80 (32)	26–137	80 (26)	40–192	90 (24)	23–190	83 (25)
Vitamin A (i.u./100 ml.)	69–148	106 (26)	84–141	112 (32)	82–158	110 (26)	75–145	109 (24)	80–138	105 (25)

Figures in parentheses indicate the number of cases.

Influence of Sex.—Our series is predominantly male, but the results show the same trend as that found by Kimble (1939) and by Moore and Leitner, in that the carotenoids are higher in females than in males, while the vitamin A is slightly lower in females than in males (Table II).

TABLE II.—*Total Carotenoid and Vitamin A Content of Blood Plasma in Males and Females*

		Total Carotenoids (μg./100 ml.)			Vitamin A (i.u./100 ml.)		
		Range	Mean	No. of Cases	Range	Mean	No. of Cases
Our series	Males	20–199	79	116	69–158	109	116
	Females	52–155	90	17	75–155	105	17
Kimble, 1939	Males	50–300	166	30	87–193	127	30
	Females	90–340	187	34	65–165	91	34
M.R.C., 1949	Males		96	46		146	46
	Females		105	37		110	37

Conclusions

Our cases were collected from patients attending eye clinics for minor accidents, for refraction, or for correction of squint. They may be regarded as a cross-section of the

population of the Midlands, having no obvious disease or chance infection. This being so, our results show that the average blood levels of the total carotenoids and vitamin A are lower in this part of England than those found in other countries.

In the Medical Research Council's Special Report on Vitamin A Requirements of Human Adults are assembled all the available data on the normal value for the carotenoid and vitamin A content of human blood plasma in 195 subjects, and Moore and Leitner, who carried out the investigation, point out that some American authors, in calculating the values for vitamin A, do not appear to have made any correction for the blue colour contributed by carotenoids to the antimony trichloride reaction. They also state that some of the earlier workers in America used the U.S.P. unit, which is now held by Hume (1943) to have only 87% of the value of the international unit, and that it is not always possible in retrospect to be certain which unit was really meant.

These facts may account for the apparent discrepancies between American and English results, as well as the fact that the people of Britain do not have access to unlimited fat, and have not acquired the taste for large quantities of green vegetables and salads. In our series 85% of cases have total carotenoid plasma levels lying between 40 and 120 μg./100 ml., and vitamin A plasma levels between 80 and 130 i.u./100 ml., and these are suggested as being reasonable limits for normality under conditions prevailing in the Midland area of England during 1947.

The results of our investigation indicate slightly lower levels for vitamin A, and considerably lower levels in total carotenoids, compared with those found by other English workers (Yudkin, 1941 ; M.R.C., 1949).

Summary

The levels of total carotenoids and vitamin A in the plasma of 133 normal adults were estimated. The investigation took place throughout the year 1947. The normal range was found to be:

Total carotenoids (μg./100 ml.)	20–199	Mean	80
Vitamin A (i.u./100 ml.)	69–158	,,	108

but since 85% of our cases gave values of

Total carotenoids (μg./100 ml.)	40–120
Vitamin A (i.u./100 ml.)	80–130

these are suggested as limits of normality under conditions prevailing in the Midland area of England in the year 1947.

Vitamin A levels showed a slight fluctuation during the year, while the level of the total carotenoids rose steadily from March to December.

Those subjects performing hard manual labour showed lower levels of total carotenoids and vitamin A than subjects engaged in light or sedentary occupations.

Thymol turbidity tests were performed on a series of 110 normal adults, and it was found that when allowance is made for a "blank" test the normal range is 0 to 1.5 ml. of barium sulphate suspension, with a mean value of 0.59 ml.

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CHLORAMPHENICOL IN TREATMENT OF INFANTILE GASTRO-ENTERITIS

A PRELIMINARY REPORT

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Bray (1945), Giles and Sangster (1948), and Taylor *et al.* (1949) independently noted a close correlation between the same serologically specific type of *Bact. coli* and epidemic infantile gastro-enteritis. This *Bact. coli* is referred to in this communication as *Bact. coli* B.G.T. (Bray–Giles–Taylor).

During the last ten months one of us (K. B. R.) has studied the appearance of this organism in 86 infants under the age of 1 year admitted to the Birmingham Children's Hospital. Routine rectal swabs were taken on admission and in many cases five days later, and again if a case of gastro-enteritis appeared in the ward. The first isolation of the *Bact. coli* B.G.T. from the stool was noted and the subsequent progress of the infant observed.

Epidemiology

In 17 cases it was possible to calculate the interval between the time of the first isolation of this organism from the faeces and the development of a gastro-intestinal disturbance. In all cases except one this period was less than 22 days ; the exception was a case which developed diarrhoea at home 24 days after the first positive rectal swab. The shortest interval was four days, in two cases ; the average interval was 11 days. It must be emphasized that in 25 of the 86 cases observed the appearance of the *Bact. coli* B.G.T. in the faeces was followed by no untoward symptoms ; these cases were followed for a period of at least one month.

This organism was isolated from the stools of 86 babies under the age of 1 year, 82 of whom acquired the organism after admission to hospital ; 25 patients, 10 of whom were less than 4 months old, had no intestinal symptoms, while the remaining 61 developed gastro-intestinal disturbances which varied from a severe gastro-enteritis to a mild diarrhoea with or without vomiting ; of these 61 patients 13 died. All of the deaths occurred in infants under the age of 4 months, except in two who were aged 10 months. One of the two, a mongoloid idiot, collapsed and died directly after discharge from hospital, but to what extent the *Bact. coli* was responsible is not known. The other, a case of pink disease, died primarily of respiratory failure associated with an extensive polyneuritis and diaphragmatic palsy, but she also had a severe terminal enteritis. The finding that *Bact. coli* B.G.T. is more commonly associated with severe gastro-enteritis in the younger babies is in agreement with the observations of Taylor *et al.* (1949). That the appearance of *Bact. coli* B.G.T. in the stools is sometimes associated with a fulminating gastro-enteritis is suggested by such typical case histories as the following.

Case 1.—A girl aged 3 months was admitted with pseudo-paralysis due to congenital syphilis, for which she received penicillin. A month after admission *Bact. coli* B.G.T. was isolated from the faeces ; 15 days later, after her transfer to another hospital for further antisyphilitic treatment, she developed diarrhoea. Four days after the onset she collapsed and died.