The Effect of Prolonged Administration of Carotene in the Form of Vegetables on the Serum Carotene and Vitamin A Levels in Man

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Few systematic investigations of the effect of prolonged administration of carotene on the blood level of carotene and vitamin A have been recorded. Kauffmann & v. Drigalski [1933] found that with continued administration of carrot juice to human subjects a maximum concentration of serum carotene (0.27 mg./100 ml.) is soon reached. Vitamin A levels were not investigated.

Wendt [1935] confirmed the occurrence of maximum values for carotene in blood in spite of continued carotene intake. The serum vitamin A, which at first rose, fell again on continued administration of carotene.

Ralli, Pariente, Brandaleone & Davidson [1936] administered various doses of 0.3% carotene in oil daily for 2-4 months to normal and to diabetic persons. With the higher doses the serum carotene rose to 0.403 and 0.680 mg./100 ml. in one normal and one diabetic subject respectively during the period of dosing, and then in the normal subject further to 0.486 mg./100 ml. during the following 7 days. There was clinical evidence of 'carotenaemia' in diabetic patients when the level had risen above 0.500 mg./100 ml. Carotenaemia was not noted in normal persons. The vitamin A levels were not investigated.

The present investigation deals with the effect of prolonged administration of different amounts of carotene in the form of vegetables on the serum carotene and vitamin A levels in healthy human subjects. The circumstance that practically all food containing preformed vitamin A is rationed favoured this experiment, since the vitamin A intake could vary only within fairly narrow limits.

EXPERIMENTAL

The serum level of carotenoids and vitamin A was established in three females and two males during periods of 14-87 days prior to the feeding period. Exp. 1 and 2 were started in March 1942 after several months of a diet comparatively poor in carotene and vitamin A. The other three experiments were done during the summer. The duration of the diets with a high carotene intake ranged from 14 to 77 days. Determinations of carotene and vitamin A in the food were not carried out, but as it seemed desirable to have figures which would reflect the dietary fluctuations in these substances, detailed records of the diets were made weekly and the total consumption of carotene and vitamin A was calculated from the data shown in Table 1. The rationed food was considered separately and the deviations from the basal spring ration (approximately 10,000 i.u./week) were plotted on the charts as fractions of the total. The higher values for the vitamin A intake are due to the greater allowances of milk and cheese during the summer. The uncertainty as to the figures for the carotene content of the vegetables is greatest with those which entered the records as 'greens'. It is indicated by the shaded areas in the diagrams, the assumption being made that the biologically available carotene content might vary between 3000 and 9000 i.u./100 g.

In Exp. 1, 2 and 4 serum was analysed which had been obtained by finger prick from fasting subjects in the morning. In Exp. 5 blood was drawn from a vein and the plasma was analysed. In

Table 1. *Relative vitamin A activity of foods*

(a) Vegetables		(b) Rationed foods	
From 'The vitamin A value of carotene in vegetables' [Graves, 1942]			
	i.u./100 g.		i.u./week
Spinach	12,000	Butter, 2 oz.	3000 [Coward, 1938]
Watercress	12,000	Margarine, 4 oz.	2000
Broccoli leaves	12,000	Milk, 3 pt.	3900
Carrots	7,500		Calculated from the above figure for butter, assuming 86.85% fat in butter [Winton & Winton, 1937] and 3.75% fat in milk [Coward, 1938]
Lettuce	3,000		[Davies & Moore, 1939]
Tomatoes	3,000	Cheese, 2 oz.	420
Brussels sprouts	1,700	Egg, 1	400 [Cruikshank, 1941]
Peas	1,000		
Cabbage	1,000		
French beans	700 [Jeghers, 1937]		

Exp. 3 and 5 the samples were taken 2-3 hr. after breakfast. Analyses were made once a week or oftener. Carotenoids and vitamin A were estimated colorimetrically as described by Hoch [1943]. The carotenoids were expressed as $\mu\text{g.}$ β -carotene and the vitamin A as i.u./100 ml. serum or plasma. Carotene and xanthophyll were not determined separately, although in Exp. 1 and 2 considerable portions of the carotenoids in blood may have been xanthophyll, due to the ingestion of spinach.

Method of extraction. The method used by Skurnik & Suhonen [1939] for the extraction of liver was employed, with modifications which prevent the presence of a protein precipitate at the beginning of the extraction with light petroleum; a heavy precipitate may impede the extraction of carotenoids and vitamin A [Yudkin, 1941]. To 0.08 (or 0.157) ml. of serum, contained in a narrow centrifuge tube (8.5 $\times$ 95 mm.) were added first 0.012 (or 0.024) ml. 40% aqueous KOH, and then, gradually, absolute ethanol to a final concentration of 30%. The air in the tube was replaced by N_2 and the tube was stoppered with a cork, fixed tightly to the tube with a loop of string, which itself was held in its place on the tube by a piece of rubber tubing. The mixture was heated in boiling water for 5 min. The tube was centrifuged to remove condensed material from the edge of the stopper before being opened, about 2 ml. of light petroleum (b.p. 40-60°) were added and the tube was then shaken for 10 min. The light petroleum was transferred to another tube of the same size with a special pipette (Fig. 1). Pipettes of this shape proved useful for the transfer of volatile fluids, the spilling of fluid from the pipette by too strong suction being prevented by the double bend. To the alkaline serum-ethanol mixture in the first tube absolute ethanol was added to raise the concentration to 50%. The mixture was again heated, under N_2 , for 1½ min. The tube was centrifuged and, after addition of about 2 ml. light petroleum, was shaken as before. The pooled extracts were washed three times with distilled H_2O , once with about 2N HCl* and again three times with H_2O . The layers were separated by centrifuging and the bottom layer was removed

Fig. 1. Pipette for transferring volatile liquids.

with the pipette which had been used for transferring the light petroleum. The extract was then dried over anhyd. Na_2SO_4 , evaporated to dryness, taken up with light petroleum and analysed for carotenoids and vitamin A.

Fig. 2 and 3 illustrate the results obtained in Exp. 1 and 2. The average intake of vegetable sources of carotene was ½ lb. of carrots/day or its carotene equivalent in other vegetables, mainly spinach (see Table 1), for periods of 63 and 77 days.

Fig. 2. Exp. 1. A.B., ♀, 24 years; $\longleftrightarrow$ period of average weekly intake of about 120,000 i.u. of carotene, mainly as carrots and spinach.

Fig. 3. Exp. 2. R.B., ♀, 24 years; $\longleftrightarrow$ period of average weekly intake of about 120,000 i.u. of carotene, mainly as carrots and spinach.

The carotene level of the serum showed an increase from 60 to about 100 $\mu\text{g.}/100\text{ ml.}$ in the period before extra carotene was given, due to increased intake of vegetables in the free diet. It then increased further to about 150 $\mu\text{g.}$ and this level was maintained during the period of carotene feeding with fluctuations parallel to the intake. On discontinuation of the surplus of vegetables the carotene level fell slightly (to 115 and 90 $\mu\text{g.}$) in Exp. 1 and 2. In Exp. 5 the plasma-carotene values varied between 120 and 160 $\mu\text{g.}$ before and during the feeding period and between 150 and 180 $\mu\text{g.}$ after the feeding period.

* In a few cases, previous to the use of HCl at this stage, turbid reactions were obtained with the SbCl_3 reagent in spite of the presence of acetic anhydride. With the procedure described here, the reaction mixture was always optically clear. The absence of the Tyndall effect was ascertained in every case.

The serum vitamin A level started to rise, apparently within the first 2 weeks of the feeding period, in Exp. 1 and 2. The average value changed in Exp. 1 from 102 before, to 147 i.u./100 ml. after, the feeding period, and in Exp. 2 from 71 to 105 i.u./100 ml. The vitamin A level remained unaltered in Exp. 5 at about 150 i.u./100 ml.

A different reaction was observed in Exp. 3 and 4 (Fig. 4), in which the diet was supplemented with 1 lb. of carrots daily for 25 and 32 days respectively. The carrots were included in the meals and consumed together with about 3% of margarine or butter. They were steamed or otherwise cooked, or (Exp. 4) grated, and steamed with the margarine for 30–40 min. The carotene level rose continuously till the 19th and 20th day to 375 and 470 $\mu\text{g.}/100\text{ ml.}$ At this time the rate of increase of the serum carotene

Fig. 4. Exp. 3. N.D.H., ♀, 29 years. Exp. 4. H.H., ♂, 33 years. $\longleftrightarrow$ period of average weekly intake of about 240,000 i.u. of carotene as carrots.

slowed down considerably in spite of continued intake of carrots for further periods of 6 and 12 days respectively. The level remained high in case 4 for a further 4 days and it fell in both cases first at a rapid rate, then more slowly. The highest levels reached were 383 and 557 $\mu\text{g.}/100\text{ ml.}$ The vitamin A levels fluctuated around 143 and 155 i.u./100 ml. during the whole experiment.

At about the time at which the initial rate of increase in the level of carotene changed, the skin of both subjects became yellow, the pigmentation, particularly of the face and hands, being intense on the 20th and 26th day respectively. The sclera showed no discoloration. The pigmentation persisted with decreasing intensity for more than 16 days after discontinuation of the carrots in the diet.

DISCUSSION

The concentration of carotene in the serum must depend on the difference between its rate of uptake into the blood and its rate of elimination, which might include chemical destruction, excretion, and deposition in stores or in tissues where it is converted into vitamin A.

The statement that with a moderate intake of carotene the serum carotene fluctuates with the intake was confirmed in the present experiments. In two cases, however, in which there was a high intake of carrots (Fig. 2), some characteristic features of the carotene curves obtained allow the following interpretation.

The increase in serum carotene was very nearly linear during the first part of the feeding period. This steady increase makes it probable that the rate of uptake into the blood, as well as the rate of elimination, was constant. The time at which the bend of the curve occurs, after the ascending part, coincides almost exactly with the visible deposition of carotene in the skin. Assuming that this deposition, together perhaps with a deposition in other less common sites, started while the other mechanisms remained essentially unchanged, then its rate must be smaller than, or, at the most, equal to, the initial rate of increase. The total amounts thus calculated to be deposited daily in these new sites are very small compared with the amounts consumed.

The bend on the descending limb of the curve, after the almost linear fall for 10 and 8 days respectively, indicates an additional output of carotenoids from stores. The slow fading of the visible pigmentation, together with the abrupt fall of the serum carotene after the maximum, suggest a much retarded release of carotene from the skin and the stores.

It is remarkable that the levels of vitamin A in these two cases, and also in one case with medium carotene intake (Exp. 5), did not rise significantly above the levels (126, 155 and 150 i.u./100 ml.) which were found at the beginning of the experiments. A significant increase in the serum content of vitamin A on feeding medium doses of carotene was observed in two cases after a period of low vitamin A and carotene intake during the winter. The original levels were 102 and 71 i.u./100 ml., and the increase seemed to have started within the first 2 weeks. The intake before the experiment thus appears not to have been optimal. The vitamin A values were in all cases within the range considered as normal.

SUMMARY

1. The intake of carotene in the form of spinach and carrots in amounts corresponding to a biological activity of 120,000 i.u./week raised the serum

carotenoid content, expressed as β -carotene, from about 100 to 150 $\mu\text{g.}/100\text{ ml.}$ in two cases, and from 120 to 160 $\mu\text{g.}/100\text{ ml.}$ in a third case.

2. The vitamin A levels rose in two cases from 102 and 71 to 147 and 105 i.u./100 ml. respectively, and remained unchanged at about 150 i.u./100 ml. in the third case.

3. Consumption of amounts of carrots sufficient to supply about twice the biological activity provided in the first three cases, resulted in a rapid rise in the serum carotene content from 120 to 375 and

470 $\mu\text{g.}/100\text{ ml.}$ within 19 and 20 days respectively. Pigmentation of the skin appeared in both cases and it was intense on the 20th and 26th days.

4. The vitamin A levels did not change significantly. The single values fluctuated in the neighbourhood of 143 and 155 i.u./100 ml. during the whole experiment.

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The Vitamin B₁ Content of National Flour and Bread—the Results of Comparative Tests by Various Methods*

A Report by

THE VITAMIN B₁ SUB-COMMITTEE OF THE ACCESSORY FOOD FACTORS COMMITTEE OF THE MEDICAL RESEARCH COUNCIL AND THE LISTER INSTITUTE

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At the request of the Ministry of Health, an investigation has been made into the merits of the various methods available for the determination of vitamin

B₁† in national flour, in order to arrive at the most suitable procedures for use in chemical laboratories where food analyses are regularly made. The present report includes a comparison of chemical with biological and microbiological assays. The vitamin B₁ content of bread made from one of the flours was also examined in order to determine the effect of baking.

The flours employed were authentic specimens of national flour, i.e. 85% extraction flour as defined in the Medical Research Council's second report on bread [Medical Research Council, 1941]. The bread was baked in a batch of 40 loaves of 2 lb. each by a usual commercial procedure. The loaves were sliced a few hours after baking and the slices placed on slatted shelves and dried at 37°. Drying was complete in 2 days when the bread, crust included, was powdered in a laboratory mill.

† I.e. aneurin, known as thiamin in the United States of America; for the purpose of this report vitamin B₁ includes aneurin as it occurs in biological materials.

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