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Absorption of Carotene from Carrots in Man and the Use of the Quantitative Chromic Oxide Indicator Method in the Absorption Experiments

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The findings of various investigators concerning the absorption of carotene from vegetables in man differ greatly. Wilson, Das Gupta & Ahmad (1937) state that only 10–20 % of the carotene from carrots or spinach is excreted in the faeces. Van Eekelen & Pannevis (1938) found 99 % of the carotene from carrots and 95 % of the carotene from spinach in the faeces. Kreula & Virtanen (1939) and Virtanen & Kreula (1941) observed a carotene excretion varying between 64 and 99 %, and generally amounting to about 90 %. The absorption from very finely grated carrots seemed to be more favourable than from carrots merely masticated. With (1940) observed with purees of tomatoes, carrots, spinach, and carrot meal, excretion values of from 40 to 70 %. Extensive experiments carried out by the Vitamin A Sub-Committee of the Accessory Food Factors Committee (Lister Institute and Medical Research Council, 1945) showed a carotene excretion of about 75 % from carrots and of about 60 % from spinach. If the carotene is dissolved in fat, a noticeably greater part of it is absorbed. Thus With (1940) observed in man excretion values of 25–70 % while the above mentioned Committee found 28 %.

METHODS

Quantitative chromic oxide method

If carotene is determined in the usual way in the faeces, the excreta must be collected quantitatively during several days and stored, frequently for a considerable time, before analysis.

In this respect the quantitative chromic oxide indicator method adopted by Edin (1926) offers noticeable advantages. This method is based on the fact that chromic oxide is not absorbed from the alimentary canal of animals and man, and is entirely excreted in the faeces. The excretion of carotene can be calculated from the proportions of carotene and chromic oxide in the food and in the faeces, and the quantitative collection of faeces is not necessary. In order to find out the excretion of carotene contained in a food, the excretion of carotene derived from the basal diet must also be taken into consideration. This can be determined in a preliminary test. If the basal diet remains unchanged in separate experiments, the basal excretion value determined for each subject may be used in a number of absorption experiments. The net excretion is determined by subtracting from the total carotene excreted during the experimental period (calculated for instance/g. of Cr_2O_3) the carotene correspondingly excreted during the basal period.

Two experiments were made to find out the suitability of the chromic oxide indicator method for the investigation of carotene absorption in man. In one a mixture of carrot and olive oil was used as a source of carotene, in the other, grated carrot alone. In the first experiment the excretion was determined simultaneously by the method involving the collection of faeces and by the quantitative chromic oxide indicator method; in the second only the latter method was employed.

Carotene determinations

In both experiments carotene was determined by the phase separation method (Virtanen & Kreula, 1941), and in the first one also by the chromatographic method according to With (1940). For phase separation, saponification was carried out under nitrogen. The procedure now used in the phase separation method differed from that in the method

previously employed in that the light petroleum (b.p. 50–80°) extract was separated in a suction flask (Jena filter 17G3) and the residue transferred to a porcelain mortar in which it was ground with 20–30 ml. of light petroleum. The manipulation was repeated twice. In general the solvent was colourless after the first treatment. The ethanolic portion of the liquid from the suction flask was combined with the ethanolic extract and the light petroleum was diluted to a fixed volume of either 100 or 150 ml.

From 10 to 15 g. carrot were used for the carotene determination and a total of 50 g. faeces with a requisite amount of sand (Virtanen & Kreula, 1941). As a rule determinations were made in duplicate except those on faeces in Exp. 1 after the first and third periods. The carotene content of the extracts obtained by the phase separation procedure was determined chromatographically.

Chromatographic analysis

The chromatographic analyses were done according to With (1940). The Al_2O_3 used was a Merck preparation standardized according to Brockmann. For analysis 5 ml. of the carotene extract in light petroleum were put through a column 10 × 70 mm. Light petroleum (b.p. 50–80°), generally a quantity of 15 ml., was also used for developing the chromatogram, the carotene passing through, and the bile pigments remaining on the column. Colorimetric determinations were done with a Pulfrich step photometer with the filter S47 and a 5 cm. cell. The percentage excretion of carotene was calculated either directly from the extinction values or from the corresponding weights of carotene calculated according to Halden & Unger (1936).

Determination of chromium

Chromium was determined in 2 g. of dry matter according to Andersen (1934), using nickel crucibles.

EXPERIMENTAL

Test subjects and diet

Three subjects took part in the experiments: H, a woman, aged 23 years and B and K, men aged 31 and 39 years respectively. For 3 days before the start of the actual experiment no carotene-containing food was eaten. The basal diet during the experiment consisted of ½ l. skim milk, ½ l. buttermilk or soured skim milk, thin hard rye bread (Swedish 'knäckebröd'), porridge cooked of rye meal, *ad libitum*, 35 g. sugar, and 50 mg. vitamin C per day per person. Water, salt, and saccharine were taken *ad libitum*.

First experiment

Experimental periods. The experiment was divided into three periods. The first period without any carotene-containing foods lasted 5 days. The faeces of the last 2 days were collected for the analysis. The second period during which carrot and Cr_2O_3 were taken began on the sixth day of the experiment and lasted 3 days. In the third period which consisted of the next 2 days only the basal diet was consumed. In both these periods the faeces were collected quantitatively in ¾ l. jars.

Additions. Raw carrot grated with a grater was mixed with olive oil and the mixture passed fifteen times through a meat grinder. The proportions were 580 g. of grated carrot and 116 g. of olive oil. At the beginning of the second period each subject consumed in one day 100 g. of the carotene-olive oil mixture containing 4·0 g. of chromic oxide (3·938 g. Cr_2O_3 by analysis). B took all at one meal, while H and K ate it in two portions.

Results. Table 1 shows the excretion of dry matter and Cr_2O_3 in the faeces. On the average 97·5 % of the chromic oxide consumed was excreted in the faeces. In calculating absorption this percentage was taken as 100.

Table 1. *First experiment. Excretion of dry matter and* Cr_2O_3

Subject	Experimental period	Faeces: Fresh weight (g.)	Faeces: Dry matter (%)	Faeces: Dry matter (g.)	Cr_2O_3 excretion: Total (g.)	Cr_2O_3 excretion: Percentage of dry matter	Cr_2O_3 excretion: Percentage of consumed Cr_2O_3
B	I	663	20·14	133·5	0	—	—
	II	1179	20·80	245·2	3·801	1·55	96·69
	III	791	21·52	170·2	0	—	—
H	I	169	23·50	39·72	0	—	—
	II	697	24·08	167·84	3·627	2·165	95·45*
	III	354	25·84	91·47	0·115	0·125	
K	I	303	25·32	76·72	0	—	—
	II	833	24·59	204·83	3·848	1·879	100·35
	III	418	22·77	95·20	0·104	0·052	

* The low value is due to an analytical error in the collecting of faeces.

The carotene content of faeces obtained by the phase separation method and by chromatographic analysis is given in Table 2.

The carrot and olive oil mixture used in this experiment was calculated by the phase separation method to contain 7·20 mg. of carotene/100 g. mixture. For a solution containing 1·0 g. of the material/100 ml. the extinction E was 0·784. By

Table 2. *First experiment. Carotene content of faeces and carotene excretion expressed as mg./g.* Cr_2O_3

				Carotene excretion					
						Chromatographic analysis			
				Phase separation method		Of extracts obtained by the phase separation		Of extracts obtained by With's method	
Subject	Experimental period	Total quantity of faeces (g.)	Dry matter (%)	Total quantity (mg.)	mg./g. Cr_2O_3	Total quantity (mg.)	mg./g. Cr_2O_3	Total quantity (mg.)	mg./g. Cr_2O_3
B	I	163	20·14	0·322	—	0·093	—	—	—
	II	1179	20·80	5·423	1·426	4·893	1·287	4·657	1·225
	III	791	21·52	0·593	—	0·127	—	—	—
H	I	169	23·50	0·118	—	0·037	—	—	—
	II	697	24·08	4·080	1·125	3·764	1·038	3·555	0·980
	III	354	25·84	0·425	—	0·446	—	—	—
K	I	303	25·32	0·212	—	0·055	—	—	—
	II	833	24·59	4·540	1·179	—	—	4·207	1·093
	III	418	22·77	0·259	—	0·121	—	—	—

extracting the pigments soluble in light petroleum directly from the ethanolic extract, an E value of 0·80 was obtained. When the 85 % acetone extraction method adopted by With (1940) was employed, the corresponding extinction was $E = 0·83$. For the chromatographic analysis the column was 10 × 70 mm. of Al_2O_3, and 5·0–6·25 ml. of carotene extract were used. As the solubility of the carotenoids in 85 % acetone seemed rather slight, an additional determination was made using at first a 96 % methanol extraction followed by extraction with light petroleum. This gave an E of 0·79. According to the chromatographic analysis the carrot material was calculated to contain 7·42 mg. of carotene/100 g. The ratio of carotene to chromic oxide in the food was thus 1·828 mg./g. Cr_2O_3 if the phase separation method was employed, and 1·884 mg./g. Cr_2O_3 if the chromatographic method was used.

The values shown in Table 3 were obtained by calculating the excretion percentages on the basis of both the method involving collection of faeces and of the chromic oxide indicator method. For subjects H and K the carotene values of the third experimental period were included in the total carotene excretion, and the values of the first experimental period were used for calculating the basal excretion. The mean values of the first and third experimental periods were used for the same purpose for subject B, and the carotene excretion was determined from the dry matter values of the second period. In calculations based on the chromic oxide indicator method the carotene and Cr_2O_3 values of the second period were used. The basal excretion was determined for H and K from values obtained in the first period, and for B from the mean for the first and third experimental periods. This could be done, since the basal excretion of carotene formed only a small portion of the total excretion during the experiment and the error probably caused in this way did not essentially affect the final result.

Calculations based on extinction values or on weights of carotene gave similar excretion percentages. As Table 3 shows, there were only slight differences between the excretion percentages calculated by the different methods. The carotene values determined from the faeces according to With (1940), with ether as solvent, were lower than the values obtained by other methods, hence also the carotene excretion percentages were lower.

Table 3. *First experiment. Percentage excretion of carotene calculated on the basis of the faeces collection method and of the* Cr_2O_3 *indicator method*

	On the basis of the collection method			On the basis of the Cr_2O_3 indicator method		
		Chromatographic analysis			Chromatographic analysis	
Subject	Phase separation method	With's method	Extraction with light petroleum	Phase separation method	With's method	Extraction with light petroleum
B	65·3	60·4	63·6	67·6	62·6	65·9
H	52·0	50·7	53·6	54·2	49·8	52·9
K	55·4	55·5	—	56·7	56·1	—

Absorption of carotene dissolved in oil. The carotene content of the oil separated by centrifugation from the carrot and olive oil mixture was found by phase separation to be 27·8 mg./100 g. The total quantity of oil consumed, 16·67 g., thus contained 4·64 mg. of carotene. On the assumption that in this experiment, as in the next one, 10 % of the carotene in the carrot itself was absorbed, and taking for the excretion percentages of subjects B, H and K respectively 65, 52 and 56, the following absorption values were obtained for the carotene dissolved in oil: B, 48·8 %; H, 69 %; and K, 62·8 %.

Second experiment

The second absorption experiment was done by the chromic oxide indicator method with finely grated raw carrots without oil. The same subjects were used. In addition to the basal diet each person took daily 100 g. of carrot and 3 g. of chromic oxide, evenly divided between three meals. The carotene content of the grated carrot, determined by phase separation, was 6·69 mg./100 g. and the carotene content of the food was thus 2·27 mg./g. Cr_2O_3.

Each voiding of faeces was analyzed separately for dry matter, Cr_2O_3 content (in 2 g. dry matter), and carotene content, the last determined in 50 g. samples of faeces by phase separation. The results of the analyses are given in Table 4.

is 2·06 mg./g. Cr_2O_3 and of subject K, 1·982 mg./g. Cr_2O_3. The ratio of carrot carotene to chromic oxide in the food being 2·27 mg./g., the following percentage excretion values are obtained: for B,

$$\frac{2{\cdot}079}{2{\cdot}27} \times 100 = 91{\cdot}6;$$

for H, 90·8; and for K, 87·3. The excretion value was calculated for H from the determinations on 18 and 19 April. The values of 17 April were erroneous since the subject had eaten plenty of vegetable-containing food before the test.

DISCUSSION

The reliability of determinations of carotene absorption depends on many circumstances. The choice of the right method for the determination of carotene is of great importance for the final result. If the source of carotene used in the experiment is analyzed by a different method than the faeces, the results are obviously not quite comparable. Some experiments performed by With (1940) suffer from this weakness. Carotene values obtained for faeces by phase separation are generally somewhat too high. This is, however, partly corrected by the higher basal excretion values. The chromatographic analysis gives the most reliable carotene values if no losses occur during the extraction of carotene and if a 'fluid chromatogram' is used.

Table 4. *Second experiment. Chromic oxide and carotene contents of the faeces*

(First dose of carotene-Cr_2O_3 was taken on 15 April.)

Date of excretion	Weight of fresh faeces (g.)			Dry matter (%)			Cr_2O_3 (% of the dry matter)			Carotene (mg./g. Cr_2O_3)		
	B	H	K	B	H	K	B	H	K	B	H	K
16. iv	*c.* 200	—	—	21·40	—	—	3·266	—	—	2·117	—	—
17. iv, *a*	344	70	140	20·49	32·35	26·91	2·790	1·162*	2·898	2·222	3·530*	2·179
17. iv, *b*	219	76	219	20·11	28·73	22·52	3·297	2·080*	3·354	1·975	2·466*	2·021
18, iv	585	320	737	18·34	26·00	19·71	2·808	4·750	2·445	2·310	2·140	—
19. iv	457	73	321	19·40	29·10	21·61	2·716	4·821	3·142	2·260	2·100	2·017
					Average		2·975	4·786	2·960	2·177	2·120	2·072

* Not included in the average.

B, Subject B; H, Subject H; K, Subject K.

Calculation of the net excretion of carotene. The net carotene excretion, for instance with subject B, calculated from the basal values of the previous experiment (Tables 1, 2), is 2·079 mg./g. Cr_2O_3, the total excretion being 2·177 mg./g. Cr_2O_3. The Cr_2O_3 percentage being 2·975 (Table 4), 1 g. of Cr_2O_3 is equivalent to 33·61 g. dry matter and to 32·61 g. chromic oxide-free dry matter, and the carotene amount excreted within this quantity is 0·098 mg. (0·30 mg./100 g. dry matter). Hence the net excretion is $2{\cdot}177 - 0{\cdot}098 = 2{\cdot}079$ mg./g. Cr_2O_3. The corresponding net excretion value of subject H

The absorption of carotene dissolved in oil seems to depend on the dose of oil as indicated by the results of the first experiment. The absorption percentage of B was 50, of H and K, 70 and 63 respectively. Determination of fat in the faeces showed, however, no apparent correlation between fat and carotene absorption.

The results show that the quantitative chromic oxide indicator method is very suitable for the investigation of carotene absorption. It gives values completely in accordance with those obtained by the method based on the quantitative collection of

faeces and by its use absorption experiments are much simplified and shortened. The faeces also need not be collected quantitatively which facilitates the treatment of the experimental material.

SUMMARY

1. The absorption of carotene was examined with three human subjects. As a source of carotene, raw finely grated carrot or a mixture of grated carrot and olive oil was used.

2. The percentage excretions were calculated by the method of quantitative collection of faeces, and also by the quantitative chromic oxide indicator method which gave the same results.

3. Of the different methods for determination of carotene, chromatographic analysis was found to be the most reliable.

4. The excretion of carotene in human beings on a fat-free diet amounted to about 90 % when finely grated carrot was consumed and to about 30–50 % when carotene dissolved in oil was taken. When the latter was taken in two portions, the excretion was 30–37 % of the carotene dissolved, when taken at one time, the excretion was 50 %.

The above work on the absorption of carotene belongs to the investigations of NJF's Kommitté för foderkvalitet (Scandinavian Society for Agronomy, Committee on the Quality of Fodder). I wish to express my sincere gratitude to the Committee for granting the means for this work.

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The Effect of Sodium Alginate on the Absorption of Calcium

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Alginic acid behaves as a monobasic acid, forming salts with a number of positive ions and its strength is comparable with that of monochloroacetic acid. Water soluble salts of Li, Na, K, Cl, Rb, NH_4 and Mg are known, and these form viscous colloidal solutions which on evaporation leave transparent films; whereas the Cu, Zn, Ag and Ni salts are insoluble in water but soluble in ammonia. The addition of Ca, Ba, Hg, Pb or Bi ions to a soluble salt results in precipitation, for salts of these metals are insoluble in water and ammonia. If these ions are released slowly from sparingly soluble substances, e.g. calcium citrate and dicalcium phosphate, continuous gels can be formed in the cold.

Because alginic acid and its salts are able to withstand any degree of heating without loss of stabilizing value or development of off flavours, this chemically reactive colloid has diverse application in the food industry to replace gelatin, agar agar, Irish moss, pectin, gum tragacanth, starch and other colloids. Solutions can be kept sterile by pasteurization for 1 hr. at 50°, or by treating with preservatives.

Alginic acid is sold in the form of its sodium salt under the trade name of Manucol. Manucol I contains 15–20 % water and a 1 % solution has a viscosity of 15–30 centistokes at 25°. Manucol IV also contains 10–20 % water but a 1 % solution has a viscosity of 80–150 centistokes at 25°. These two products are used widely as stabilizing agents in ice-cream, reconstituted and artificial creams, chocolate milk suspensions, marsh-mallows, fruit squashes, and as thickening agents in custards, jams, marmalades, sauces, soups and water jellies.

Owing to their increased application in the manufacture of foods, it was considered necessary to investigate the possibility of alginic acid or alginates interfering with calcium absorption in man. Calcium alginate is stable to a pH of 3·9, below which the calcium ion is released and the