The Relationship between Carotene Concentration and Vitamin A Activity of Carrots for Rats

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The vitamin A values of plant foodstuffs, as found by biological tests with rats, are often lower than would have been expected from the results of chemical estimations of the carotene content. The causes of this discrepancy presumably lie mainly in a less complete absorption of carotene from vegetables than from the oily solution in which the international β-carotene standard is administered, and in the inability of the chemical methods, as commonly applied, to discriminate between β-carotene and isomers of lower biological activity. The present paper is concerned with the experimental evaluation of the ratio between biological and chemical values for carrots.

Mode of expressing the biological and chemical results in units

The international unit of vitamin A is defined as the vitamin A activity of o·6 μg. of pure β-carotene (League of Nations, 1934). When the carotene content has been determined by chemical means the results are most commonly expressed by weight in mg. or μg., but when the vitamin A potency has been determined by biological means the quantities are usually expressed in international units (i.u.). As this paper is concerned with the numerical relationship of the values obtained by the two methods, it is convenient for purposes of comparison to express the chemical values also as i.u. This is done by dividing the number of mg. of carotene found chemically by the value 0·0006, but, in order to distinguish the results of chemical tests expressed in this way from the results of biological tests, the term 'ch.i.u.' will be used in the former case.

The relationship between biological and chemical values has in the past usually been expressed as the chemical/biological ratio, i.e. as ch.i.u./i.u. The biological value observed may, however, vary with the technique according, for instance, to whether rats or other animals are used, to the method of preparation of the diet and supplement, and to whether growth or some other criterion is selected for measurement. The chemical value, on the other hand, can be determined with much more accuracy. Therefore, it seems more rational to express the biological value as a percentage of the chemical value, i.e. i.u. × 100/ch.i.u. This is a similar relationship to that called the 'biological value' by Guggenheim (1944).

Values previously reported for the vitamin A potency of carrots

Results of other workers for biological determinations of the vitamin A value of carrots appear in Table 1. The values of Scoular, Bellew, Carl & Dozier (1943), and of Wilson, Ambrose, DeEds, Dutton & Bailey (1946) were based on the dry weight.

To make these values comparable with the others in the table they have been divided by 8 on the assumption that the fresh carrot contains 87·5% of water. The doses used by Coward & Morgan (1935) were high; their value for August carrots of 1900 i.u./100 g., the lowest in the list, is consequently only a minimum value. From the limited data of Smith & Otis (1941) values of 10,000 for pale orange, and 17,000 for red, carrots may be obtained by calculation; their experiments do not, however, appear to have been done under standard conditions; there was, for instance, only a small number of rats in certain groups.

Table 1. *Values reported by other workers for the vitamin A activity of carrots determined biologically, with values obtained chemically for the same material where such tests were also made*

	Carotene content/100 g. fresh wt.			
Authority	Determined biologically (i.u.)		Determined chemically (ch.i.u.)*	Biol. value × 100 / Chem. value
Coward & Morgan (1935)		1,900	—	—
Hanning (1936)		6,500	—	—
Scheunert & Wagner (1940)		10,000	8,000	125
Booher, Hewston & Marsh (1941)		10,700	—	—
Smith & Otis (1941)	Yellow carrots 10,000† Red carrots 17,000† }	13,500†	2,000 23,000 } 12,500	108
Sherman, H. C. (quoted by Graves, 1942)		2,500	—	—
Scoular, Bellew, Carl & Dozier (1943)		2,500†	—	—
Wilson, Thomas & DeEds (1943)		20,500	16,800	122
Guggenheim (1944)		7,100	12,500	57
Oser & Melnick (1945)	6,400 3,600 }	5,000	10,300 5,040 } 7,670	66
Callison & Orent-Keiles (1945)		7,000	—	—
Wilson, Ambrose, DeEds, Dutton & Bailey (1946)		19,200†	21,700	88
	Mean 8,870			94

* For explanation see p. 113.
† Calculated from data in the authors' papers.

American usage prefers cod-liver oil to β-carotene as standard for vitamin A. According to evidence from various sources, summarized by Morton (1942), the U.S.P. reference cod-liver oil has lost part of its activity: hence biological values determined against it are likely to be high. Confirmation comes from Callison & Orent-Keiles (1945) who have made both comparisons: the average result of four bio-assays on carrots against the U.S.P. cod-liver oil was 11,000 i.u./100 g., and against β-carotene 7000. The authors give the mean of the differences by which the results obtained against the reference oil are too high as 34% but, if the calculation is made on the basis of the values obtained against carotene as standard rather than of that obtained against the reference oil, the difference is 54%. Since some of the values in Table 1 were obtained against the U.S.P. reference oil they must also presumably be too high in varying degree according to the date of the assay.

Another probable cause of error in biological tests· on carrots is that the basal diet has not always included vitamin E, although this is a dietary essential for the rat and is known to play a part in vitamin A metabolism and to influence the requirement (Moore, Martin & Rajagopal, 1939; Hickman, Harris & Woodside, 1942; Guggenheim, 1944; Moore, 1945).

Seasonal variations in carotene content have not always been considered. According to Barnes (1936), Werner (1940), and Booth & Dark (unpublished), maturity is not reached until autumn. Carotene values in early August, for instance, may be only 50–80% of the mature values. Consequently a biological value, unaccompanied by a description of the carrots including the date of harvesting, or by a chemical value, has little meaning.

The average of all the biological values in Table 1 is 8870, but in view of the above considerations its accuracy is doubtful. In six cases only are values given by the same authors for results obtained by both chemical and rat-growth methods. The average biological/chemical percentage for these cases is 94. On the other hand, Fraps & Meinke (1945), using a liver-storage method, found the percentage to be 30. Graves (1942) suggests that the chemical/biological ratio may be as high as 6, so that the biological/chemical· percentage would be 17. Smith & Otis (1941) report a wide variation with carrots of different colour.

For the carotene content of carrots determined chemically, more than forty references have been found in the literature. The average value is 9 mg. (15,000 ch.i.u.)/100 g. but, because of immaturity and inappropriate methods of assay, this value is too low (for discussion see Dark & Booth, 1946). However, for comparison with the average of the published biological values, it seems appropriate to use it to compute a percentage. This is $8870 \times 100/15,000 = 59\%$; the degree of accuracy is unknown but, judged by the results described below, this value is nearer the truth than the value of 94% mentioned above, obtained from the results of various direct comparisons.

<div align="center">METHODS</div>

<div align="center">*Determination of vitamin A potency by the rat-growth method*</div>

Rats of both sexes were weaned on to a vitamin A-free diet having the percentage composition: extracted casein (Glaxo C/E or A/E) 17, arachis oil 13, sucrose 53, dried yeast 13, salt mixture 4. Each rat received also 2 mg. racemic α-tocopheryl acetate mixed with 40 mg. (120 i.u.) of Radiostol (synthetic vitamin D of the British Drug Houses Ltd.) once a week. From about 25 days of age the rats were kept in individual cages and weighed at least twice weekly. Immediately they had ceased gaining weight they were given graded doses of cooked or raw carrot or of international standard carotene. Two groups received 5 and 7·5 i.u. standard carotene, freshly diluted with arachis oil containing 0·01% quinol, twice a week. The oil was given directly into each rat's mouth from a dropping pipette. Three other groups were given closely graded doses of various specimens of carrot predicted to give a response approximately equal to that given by the two doses of standard. For an average carrot these were 16, 22 and 30 ch.i.u. carotene for each of the two doses per week,

less for pale carrots, and more for deep red ones, since the carotene in the latter proved to be less well utilized. Experience has emphasized the necessity for the doses to be closely graded because accurate computation of the result is only possible if the responses to the standard and to the unknown overlap. Growth rates during 4 weeks of dosing were calculated from growth curves. The averages for males and females were calculated separately, and the means of these pairs of averages were used for plotting dose-response curves. One or more of the doses of carrot always gave a growth response close to or between the responses to the two doses of standard. The activity was calculated by direct comparison of the dose of carrot giving the same response as an interpolated dose of standard.

Because cut carrot loses water to air, it is important that sampling and weighing should be done rapidly. The total supplement for a group of rats was, therefore, weighed and then divided into similar sector-shaped pieces, the doses being equalized by inspection. Non-uniformity would be negligible when spread over the eight doses for the experimental period. At the same time, sectors were cut and weighed for chemical analysis.

For tests with cooked carrot the pieces for both biological and chemical tests were placed in separate test-tubes, which were immersed in boiling water for 45 min. A few drops of water were added where appropriate to counter desiccation.

For the chemical and biological tests with raw carrots, the samples were prepared in the same way but without cooking.

Estimation of vitamin A potency by the liver-storage method

This technique was used as a confirmatory one. The procedure was broadly similar to that described by With (1942), Guggenheim (1944), Fraps & Meinke (1945) and others, except that the doses were larger and were given over a longer period. Rats depleted of vitamin A were divided into groups which were matched as far as possible in litter, sex and weight. Each group was given carrot, sampled as described above, or carotene in oil, on 6 days a week for 3 weeks. Two days after the last dose was consumed the rats were killed with coal gas, and the vitamin A content of each whole liver was determined by the method of Davies (1933) (see also Davies & Moore, 1939). With a dose of 0·8 mg. (1330 ch.i.u.) β-carotene, storage was negligible under the conditions of the present experiment, so that this was the minimum dose suitable for the purpose. Females stored more vitamin A in relation to the dose than males. The average for each sex was, therefore, worked out separately, and the means of these averages were used in calculating all results.

Estimation of carotene excreted by rats in their faeces

Rats on the vitamin A-free diet were kept in individual cages with wire-grid bottoms beneath which the droppings were collected on sheets of paper. Supplements were given as described above. The faeces from each rat were softened with water and extracted with a mixture of light petroleum and acetone in the proportion of 3 : 2 by volume, next with acetone and then several times with the mixture. From the combined extracts the acetone was washed with water, leaving fats and pigments in

the light petroleum. The fat was removed by cold saponification and washing. The carotene was freed from other pigments with aqueous diacetone alcohol or on alumina-Na_2SO_4 and the amount was determined in the photometer (Booth, 1945a).

Small amounts of a carotene-like pigment are always found in faeces even after prolonged subsistence on a carotenoid-free diet (see for instance Kemmerer & Fraps, 1938; Ramasarma & Hakim, 1942; Guggenheim, 1944). Several experiments have been done by the present author to evaluate this residual pigment or 'blank'. It was found to be approximately proportional to body-weight and averaged 1·1 and 0·8 μg. 'carotene'/24 hr. for male and female rats, respectively, at a body-weight of 200 g. The amount of this residual pigment was subtracted from the total carotene recovered from the faeces, and the percentage of the ingested carotene which had disappeared was calculated separately for each rat. In none of the experiments in which both bucks and does were used was there any significant sex difference in this percentage disappearance. Accordingly, it was deemed safe to weight experiments on females only equally with those on both sexes.

Up to 3 days are required for passage of unabsorbed food from mouth to anus in the rat. Usually no food carotene could be detected in the faeces later than 60 hr. after the meal but, if the dose contained over 400 μg., small amounts were sometimes detected 72 hr.. afterwards. Hence faeces had to be collected for long enough to recover all the excreted carotene, but not for longer than was necessary, because of the progressive increase in the correction needed for the 'blank' or residual carotene. A suitable period for most ordinary intakes was from a few hours after dosing until 65 hr. after the dose, or after the second dose if two were given. Since the loss of carotene in stored faeces was found to be only 17 % in 15 days at room temperature, it was assumed that loss during the period of collection was negligible. The possibility of destruction within the alimentary canal was not of course excluded by this finding.

Chemical determination of carotene content of carrots

The carrots were assayed chemically for total carotene, most of it β- and α-carotenes, by the sector method (Booth, 1945b). Carotenols (xanthophylls) were removed in some cases with diacetone alcohol, in others with alumina-Na_2SO_4 mixture. No significant difference was found when both methods were used with carrot extracts. The same carrots as were given to rats were also tested chemically on each dosing day. Colorimetric determinations were done on the Pulfrich gradation photometer calibrated with the international standard carotene, by which means a true comparison was obtained between biological and chemical values. In selected cases the carotene was separated into its two major constituents, the β- and α-groups, by first determining the content of total carotene with the alumina-Na_2SO_4 technique, then washing out the acetone from the light petroleum solution, and separating the pigments on magnesium oxide.

Test material

The carrots (*Daucus carota* L.) used were grown on various soils in south-east England. They were of three types, classified according to their concentration of carotene. (1) Very young, immature, yellow roots, weighing only some 8 g. each and

having about 3·3 mg. (5500 ch.i.u.) carotene/100 g. fresh weight. (2) 'Ordinary' carrots of the commonly grown varieties having at maturity about 9–18 mg. (15,000–30,000 ch.i.u.)/100 g. The average value has been found to be 12·4 mg. (Dark & Booth, 1946). (3) The exceptionally rich red carrots of the long varieties, bred and described by Dark & Booth (1946), having about 50 mg. (80,000 ch.i.u.)/100 g.

RESULTS

Results of the rat-growth tests in relation to the results of the chemical tests

Parallel determinations of carotene content were made by the rat-growth method and by the chemical method on ten samples of carrots having a wide range of carotene values. The results are shown in Table 2. The dates show to the nearest month when

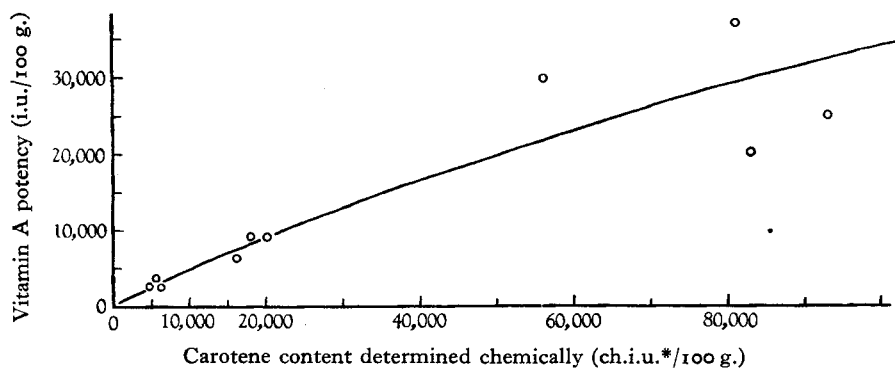Carotene content determined chemically (ch.i.u.*/100 g.)

Fig. 1. Relationship between the carotene content of carrots determined chemically and the vitamin A potency determined biologically by growth tests with rats.

* For explanation see p. 113.

dosing was in progress. In some cases carrots were freshly dug for a test; in others they were lifted during the winter, stored in burnt soil for a month and then in a closed container in the refrigerator. Under these last-named conditions carrots can be kept healthy for many months with only negligible change in weight.

The limits of error in most of the bio-assays are 65–155 % with $P=0.95$. It is evident from inspection of Fig. 1 that the results are well within these limits. Exact computation of the error is impossible because of a deterioration of the international standard carotene, which was believed to have taken place during the experiment (see Discussion, p. 123).

The values obtained by biological assay expressed as percentages of the chemical values are shown in the eighth column of Table 2. The variations in this percentage are presumably due to differences in the experimental conditions caused by the variety, mode of cultivation, age and state of the carrots, whether they were cooked or raw, in the proportion of β-carotene they contained, in the season of the year, in the freshness of the standard, and in the responses of the rats. The number of experiments, however, justifies the construction of the curve shown in Fig. 1. The relationship between the values for vitamin A potency obtained by growth tests and the carotene values obtained by chemical tests is not quite linear, the biological/chemical percentage

Table 2. *Vitamin A potency of carrots determined chemically and by rat-growth tests*

Variety	Condition	Date of dosing	No. of rats	Carotene content/100 g. fresh carrot			Biol. value × 100 / Chem. value	
				Determined chemically (ch.i.u.)†	Determined chemically and corrected for α-carotene* (ch.i.u.)†	Determined biologically (i.u.)	Observed	Corrected for α-carotene
Chantenay	Fresh, cooked	July 1942	25 P	4,900	3,970	2,900	59	73
S.R. Intermediate	Fresh, cooked	Aug. 1943	61 P	5,600	4,540	3,900	70	86
Chantenay	Fresh, cooked	July 1942	41 P	6,250	5,060	2,800	45	55
James's Sc. Int.	Fresh, raw	Nov. 1941	50 A	16,100	13,000	6,500	40	50
Chantenay	BS, F, raw	Feb. 1945	40 P	18,000	14,600	9,200	51	63
Chantenay	F, cooked	Apr. 1942	40 P	20,200	16,400	9,200	46	56
L.R.S.R. Coreless	BS, F, raw	Mar. 1942	38 A	56,000	45,400	30,000	54	66
Altrincham	BS, F, cooked	Feb. 1943	44 P	81,000	65,600	37,000	46	56
Surrey Long	BS, F, cooked	July 1943	45 P	83,000	67,000	20,100	24	30
Altrincham	BS, F, cooked	Mar. 1943	35 P	93,000	75,300	25,000	27	33

* The correction for α-carotene is made by taking 81 % of the value in the preceding column (see p. 123).
† For explanation see p. 113.
A = albino rats, males only. P = piebald rats of both sexes. BS = stored in burnt soil for a month. F = stored in refrigerator.

falling slightly with increasing concentration of carotene in the roots. To take an extreme instance, an exceptionally rich carrot having 100,000 ch.i.u./100 g. with a vitamin A activity of 34% has not 10 but only 7·1 times the biological value of an unusually poor carrot having 10,000 ch.i.u./100 g. and a vitamin A activity of 48%.

Liver storage of vitamin A in relation to the type of carrot consumed

In order to confirm the existence of the variation just mentioned in the biological value of carotene with the variation in its concentration within the carrot, a direct comparison was made between 'ordinary' and 'high-carotene' carrots by the liver-storage technique. In four experiments 140 piebald rats were dosed with carrot and then killed. In Fig. 2 the average vitamin A content of the livers (estimated in-
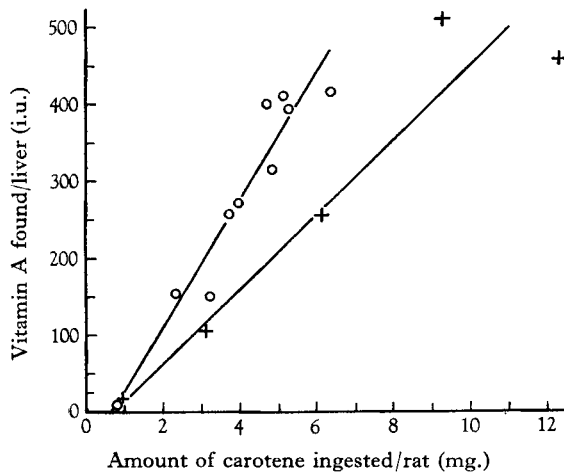

Fig. 2. Relationship between the amount of carotene eaten and the amount of vitamin A found in the livers of rats. ○ Carotene from carrots with about 11 mg./100 g; + carotene from carrots with about 50 mg./100 g.

dividually) is plotted against the dose of carotene ingested for the two types of carrot. Each point represents the average of the values for a group of several male and female rats. The dose-response curves are straight lines. The slope of the line for ordinary carrots having a carotene content of 11 mg. (18,300 ch.i.u.)/100 g. is 1·7 times as steep as the slope of the line for high-carotene carrots with 50 mg. (83,000 ch.i.u.)/100 g. The carotene in the less rich carrots was, therefore, 1·7 times as effective for promoting the storage of vitamin A as the same amount of carotene from the richer carrots. The corresponding relationship between these two types of carrot, computed on the basis of the growth tests from the curve in Fig. 1, is 1·4. The liver-storage results, therefore, support the growth results; the agreement is not exact, but it is noteworthy that the amounts of carrot ingested in the storage experiments were nearly 100 times those ingested in the same time in the growth experiments. In the latter the intake was necessarily suboptimal and the rats were to that extent abnormal, while in the former a rat may have had to consume nearly half its own weight of carrot in 3 weeks.

When tested by the liver-storage technique, carotene in ordinary carrots had about

the same vitamin value as it had in oil. This fact may appear to be inconsistent with the results of the growth tests. The explanation, which is outside the scope of the present work, appears to be connected with the great size of the doses and the consequently increased intake of accessory factors in small amount. The comparison of one carrot with another by the liver-storage method is, however, unlikely to be affected; if it were, the dose-response curves would not both be straight lines.

Intestinal absorption of carotene from oil and from different kinds of carrots

When food containing carotene is ingested, some carotene can always be recovered in the faeces. The carotene which disappears during the passage of food through the gut is usually assumed to have been absorbed but, until this is proved, the term 'disappearance' seems preferable to 'absorption'. Moreover, it is generally supposed that the smaller absorption of carotene from carrots than from oil explains the lower biological value of carotene in the former.

Table 3. *Some published results of studies on the 'absorption' of carotene by rats from carrots and from oil*

Authority	Source of carotene	Proportion of ingested carotene not excreted (%)
Wagner, Gunther & Schmalfuss (1943)	Carrot	19–38
Iljina (1943)	Carrot	46
Guggenheim (1944)	Carrot	50
Wilson, Ahmad & Majumdar (1936)	Oil	74
Kemmerer & Fraps (1938)	Oil	51
With (1940)	Oil	77
Wagner, Günther & Schulze (1941)	Oil	70–94
Ramasarma & Hakim (1942)	Oil	87
Guggenheim (1944)	Oil	80

There are various reports in the literature of studies on the alimentary absorption of carotene by rats. Some of them are set out in Table 3. The average percentage absorption of carotene was 42 from carrots and 76 from oil. The proportion of carotene which disappeared from carrots was thus only 55% of that which disappeared from oil. In most of these studies no special care was taken to ensure that the intake of vitamin E was adequate. It seemed advisable, therefore, to check the validity of the relationship thus arrived at, by using rats sufficiently supplied with vitamin E.

A group of twelve rats was given doses of carrot containing varying amounts of carotene over the range from 9 to 640 μg. The percentage which disappeared was greatest from small doses. This fall in efficiency of absorption with increasing dose, at the low end of the range, was several times confirmed. The accuracy with low doses is, however, seriously impaired by the relative size of the 'blank' correction. For this reason in most of the experiments the doses given were large enough to reduce the blank to insignificance. These doses were much higher than those used for the growth tests but, since the prime object of this series of experiments was to compare carotene in carrots with carotene in oil, it was essential that the dose levels for the two should be comparable.

The results of several such experiments are summarized in Table 4. It will be seen that much the greatest amount of carotene disappeared from oily solution, the average being 73%. From pale yellow carrots the efficiency of absorption was greater than from the deep red, high-carotene type. Cooked carrots were a little better than raw, but the difference was not great. For 'ordinary' carrots containing 12·4 mg.

Table 4. *'Disappearance' of carotene during the passage of food through the alimentary tract of rats*

Source of carotene	Concentration of carotene in carrot (mg./100 g.)	No. of rats	Total amount of carotene eaten (μg.)	Proportion of carotene disappearing (%)	s.d.	Carrot × 100 / Oil
Carrot, raw	7·0	8 ♀	158–216	56	10·3	77
Carrot, raw	9·3	11 ♀	196–440	56	5·8	77
Carrot, raw	13·2	12	9–640	47	*	64
Carrot, raw	20·2	3	202	37	—	51
Carrot, cooked	20·5	19	54–1120·	41	6·9	56
Carrot, raw	66·0	11 ♀	296–1650	33	10·5	45
Carrot extract in arachis oil	—	2	208	72	—	—
β-Carotene in arachis oil	—	22	199	73	4·7	—
International standard carotene in coconut oil	—	5	7	78	—	—

Weighted mean for carotene in oil 73

* The percentage varied with the dose level; see p. 121.

carotene/100 g. the disappearance was calculated, from a curve constructed from the data in Table 4, to be about 47%. The proportion of carotene absorbed from ordinary carrots was thus 64% of the proportion absorbed from oil. Table 4 shows the standard deviation of the mean percentage disappearance in each case in which the number of animals in a group justified its calculation. The mean of the standard deviations, weighted by the number of animals in a group, was ±6·94.

DISCUSSION

Variation in the biological/chemical percentage according to the type of carrot

The results obtained by the various methods agree in showing that the biological value for rats of the carotene of carrots varied (but in opposite sense) with the concentration of carotene in the carrot, though the variation was small within the range of concentration of ordinary carrots. If the early varieties are included, the limits for the biological/chemical percentage are approx. 42–52. Differences in digestibility might account for a part of the variation since the less rich carrots were young and fresh, while the richer ones were old and stored.

Contribution made to the biological activity by α-carotene

In this paper 'carotene', which is generally 90% of the total carotenoids, means the complete mixture of β-, α- and γ-carotenes and their *cis* isomers as it occurs in carrots, but excludes carotenols. According to Harper & Zscheile (1945) the β-isomer

represents 54% of the carotene; Sadana & Ahmad (1947) found 56%; Mackinney & Fratzke (1947) 58%; Kemmerer & Fraps (1945) 60%; Fujita & Ajisaka (1941) 61%; Kemmerer, Fraps & Meinke (1945) 62%; and Broge (1945) 76%. We have found well over 60%. The average is, therefore, unlikely to differ much from 62%. The remaining 38% is principally α-carotene but includes small amounts of other isomers. Wilkinson (1941) found that α-carotene from palm oil had half the biological activity of β-carotene. If the vitamin A activity of the above-mentioned 38% is taken as half that of the same amount of β-carotene, the activity of the total carotene from carrots should be 81% of the activity of the same amount of β-carotene. In order to express the values for total carotene in terms of β-carotene, the chemical and biological values in columns 5 and 8, respectively, in Table 2 have been recalculated on this basis, and the results appear in columns 6 and 9. Elsewhere throughout this paper the observed values for total carotene have not been so corrected.

Part played by the inefficiency of intestinal absorption

The lower activity of α-carotene accounts only partly for the divergence of the biological/chemical percentage for carrots from 100. The remainder of the divergence is generally assumed to be due to lower alimentary absorption of carotene from plant tissues than from oil. For ordinary carrots of the type containing 12·4 mg. (20,700 ch.i.u.)/100 g. the biological/chemical percentage derived from Fig. 1 is 46·5. The correction for α-carotene raises the percentage to 57. The absorption (as assessed by alimentary disappearance) of carotene from ordinary carrots was only 64% of the absorption of carotene from oil. These two percentages may be compared. The first represents utilization, and the second absorption, of carotene from carrot in terms of carotene from oil. The values (57 and 64) are sufficiently close to warrant the statement that we can account for most of the difference between chemical and biological values for carotene in carrots, that is, for the low utilization of the carotene in the latter. Whether the remaining 7% is covered by experimental error or by a third factor cannot at present be settled. It is noteworthy that the values for the biological/chemical percentage, the liver storage and the efficiency of absorption, all rise when the concentration of carotene in carrots is low. No doubt the last of these controls the other two.

Deterioration of the international standard solution during the experiments

Reference has been made to a loss of activity by the international standard carotene which took place during the work. Every precaution was taken in storing the standard carotene and using it in a fresh condition, but the colorimetric values suggested that most samples had suffered some loss. The occurrence of this destruction was established by direct comparison with pure β-carotene and by adopting for the colour test a value of 2550 for $E_{1\,cm.}^{1\,\%}$ at 450 mμ. in light petroleum, arrived at after consideration of data of Devine, Hunter & Williams (1945). The average amount of deterioration was about 10%. In the present work the same samples of international standard carotene were used both for chemical and biological assays, so that the small amount of deterioration in the standard would scarcely affect the observed biological/chemical percentage.

The true biological values of carrots, if estimated against fresh pure β-carotene, would, however, be proportionately lower than the values reported here, and each biological/chemical percentage, therefore, really belongs to a slightly lower carotene concentration. In other words, the scale for both the chemical and biological values in Fig. 1 ought to be expanded, or the curve ought to be contracted downwards and towards the left, but, since the shape of the curve would be unaffected, its usefulness remains. In the practical application of the curve this effect makes very little difference, as the following example shows. From Fig. 1 it may be seen that the biological value for ordinary mature carrots of commonly grown varieties having 12·4 mg. (20,700 ch.i.u.) total carotene/100 g. is 9600 i.u./100 g., and hence the biological/chemical percentage is 46·5. The result of a 10 % contraction of the curve at a true, chemical value of 20,700 ch.i.u. would be to shift the percentage from 46·5 to 46·0 and the biological value from 9600 to 9500. This small change is of course due to the fact that the curve is not linear. Even had the standard deteriorated by more than 10 % it seems unlikely that the percentage at this level would be less than 44. There is also the possibility that a loss of potency of the standard might have occurred through trans → cis isomerization. Taking this into consideration the biological value suggested for mature carrots is 9000 i.u./100 g.

Application of the results to human diets

The results in this paper are derived from experiments on rats, and the same is true of the vitamin A values attributed to most foods. The possibility exists, however, that carrots have a different relative vitamin A value for man.

SUMMARY

1. The values found in the literature for the carotene content determined chemically, and the vitamin A potency determined biologically, in mature carrots were inadequate for determining the ratio of these two properties to one another.

2. In the present paper, this ratio is called the biological/chemical percentage, and is defined as one hundred times the biological value divided by the chemical value.

3. The carotene content was determined chemically, and the vitamin A potency biologically by the rat growth method, against the international standard solution of β-carotene for ten samples of seven varieties of carrots.

4. The carotene content determined chemically ranged from 4900 to 93,000 ch.i.u.[*], and the biological value from 2900 to 37,000 i.u./100 g. wet tissue. The biological value tended to be less in proportion as the concentration in the carrot increased, the biological/chemical percentage ranging from 24 to 70; for ordinary carrots with a concentration of carotene of 12·4 mg./100 g. this percentage was 46.

5. A curve was constructed from which may be read the vitamin A potency, for rats, of any carrots having a known carotene content determined chemically.

6. The existence of an inverse relationship between the biological potency and the concentration of carotene in carrots was confirmed by measuring the amount of

[*] For explanation see p. 113.

vitamin A stored in the liver of rats dosed with two types of carrot, one rich, and one poor, in carotene.

7. The amount of carotene excreted by rats in their faeces was determined and the percentage of ingested carotene which disappeared (and was presumably absorbed) was calculated.

8. The disappearance of ingested carotene ranged from 33 to 56% when it was derived from carrots, being least when the concentration in the carrot was greatest; for ordinary carrots it was 47%. When the carotene was derived from oily solution the disappearance ranged from 72 to 78%.

9. The difference between the biological and chemical values can be almost wholly accounted for by the incompleteness of alimentary absorption, and by the lower activity of α-carotene which forms an appreciable part of the total carotene in carrots.

10. The biological value suggested for the vitamin A potency of ordinary carrots, determined in experiments on rats, is 9000 i.u./100 g.

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Haemoglobin Levels in Pregnancy

The Effect of the Rationing Scheme and Routine Administration of Iron

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It has been a medical commonplace for many years that women frequently suffer from a mild degree of anaemia during pregnancy. The anaemia is often referred to as 'physiological' and thought to be associated with the hydraemia which has been shown to occur at this time (Dieckmann & Wegner, 1934; Thompson, Hirscheimer, Gibson & Evans, 1938). This theory of a 'physiological' anaemia of pregnancy was based on work on hospital patients who, broadly speaking, were seldom in optimal health, and for some time we had considered the possibility that in the really well-fed woman this anaemia might be absent or negligible. Wartime conditions, by the introduction of the general rationing scheme and particularly by the introduction of special rations for pregnant women, had improved the diet of expectant mothers, especially those of the hospital class. But, though the diet of these women had improved by the end of 1943, it was still considered low in iron judging by the number of pregnant women with hypochromic anaemia. In order, therefore, to investigate this question of anaemia in pregnancy further, an experiment was started at the end of 1943 planned to show the effect on the haemoglobin level and on the general health of the pregnant woman, not only of the improved rations but also of the routine administration of iron. At the same time a fuller blood examination and serial serum-protein estimations were to be done on some of the cases. Unfortunately, a flying bomb incident interrupted the work, but, though the actual numbers of observations were not as large as expected, they were large enough for statistical analysis and are set out briefly in the present paper.