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The Biological Value of Carotene from Various Sources and the Effect of Vitamin E on the Utilization of Carotene and of Vitamin A

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In 1934 the Second International Conference on Vitamin Standardization defined the international unit of vitamin A as the vitamin A potency of 0.0006 mg. of β -carotene. In the last decade, however, it has become clear that in many cases a simple relationship does not agree with the findings. In experiments with human beings (Booher & Callison, 1939; Booher, Callison & Hewston, 1939) and with animals (Guilbert, Howell & Hart, 1940; Smith & Otis, 1941; Treichler, Kemmerer & Fraps, 1942) it was found that vitamin A was utilized better than carotene. Moreover, several authors (Dyer, Key & Coward, 1934; Kraybill & Shrewsbury, 1936; Lease, Lease, Steenbock & Baumann, 1939; Sherman, 1941; Treichler *et al.* 1942) pointed out that the utilization of carotene by rats was influenced by the nature of the oil used as solvent. It appears, therefore, that the resorption and transformation of carotene into vitamin A depends on factors of which the nature and mechanism are as yet unknown.

It seems, therefore, that existing evidence is contrary to the assumption that 0.0006 mg. β -carotene always has the biological potency of 1 i.u. vitamin A. Its efficiency as pro-vitamin A depends, apparently, on many factors intimately connected with the material in which it is contained (various oils, plant materials). A more suitable descriptive term of the quantitative biological potency of carotene would

be the *biological value of carotene*, which may be defined as:

$$\frac{\text{Actual vitamin A potency of the material, expressed in terms of i.u.}}{\text{Carotene content of the material, determined chemically, expressed in terms of i.u.}} \times 100.$$

The liver storage test, described in a previous communication (Guggenheim & Koch, 1944), served as a useful means for assessing vitamin A potency. Using this method, we have determined the biological value of carotene of various human and animal foods.

METHODS

For each examination, 8 or 10 test rats and an equal number of control rats were used, each group comprising an equal number of males and females. The preparation of the rats for the experiments, the vitamin A-free diet, the method of vitamin A determination in the rat livers, and the experimental procedure have been described in a previous paper (Guggenheim & Koch, 1944). The test rats and the control rats were given on two successive days the material to be tested or a measured amount of vitamin A respectively. On the fourth day the rats were killed and their livers examined. In the present study 'Prepalin' (Glaxo Laboratories Ltd.) was also used as a vitamin A standard preparation. The respective amounts of carotene and vitamin A of the materials given to the test rats, and that of vitamin A given to the control animals, were chosen according to their body weight. Carotene was determined according to the

method of Guilbert (1934) as modified by Peterson, Hughes & Freeman (1937).

The method of determining the biological value of carotene is illustrated by this example. A sample of carrots which contained 7.5 mg. of carotene/100 g. was tested on 10 test rats and 10 control rats, weighing 42-49 g.; the average weight of each group was 46 g. The control rats received 46 i.u. vitamin A each, i.e. 10 i.u./10 g. body weight, on two successive days; the test animals were given 1 g. of carrots = 0.075 mg. carotene, assumed to be equal to 125 i.u., or 27.2 i.u./10 g. body weight. The average amount of vitamin A recovered from the livers of the control rats was 6.0 i.u., and that from the test animals, 13.2 i.u. According to our curve (see Guggenheim & Koch, 1944), 6.0 i.u. recovered correspond to 11.2 i.u. given, i.e. in this experiment the reaction of the control rats was the same as if they had been given 11.2 i.u./10 g. body weight instead of 10 i.u. The 13.2 i.u. recovered from the livers of the test rats which received carotene correspond on our curve to 17.4 i.u. administered. From the equation

$$\frac{x}{10} = \frac{17.4}{11.2}, \text{ or } x = 15.5,$$

where x equals number of i.u. given to the test rats/10 g. body weight, and 10 equals the number of i.u./10 g. body weight of vitamin A given to the control rats, we find that the vitamin A potency of the carotene contained in the carrots fed to the test rats is 15.5 i.u./10 g. body weight. The amount of carotene determined chemically was, however, 27.2 i.u.; hence the biological value of carrot carotene is

$$100 \times \frac{15.5}{27.2} = 57.$$

RESULTS

The biological values of plant materials and their carotenoid content. Table 1 presents the biological values of vitamin A and carotene in the various foodstuffs fed to the experimental rats. The biological value of the vitamin A preparations 'Navitol' (E. R. Squibb and Sons), which contains vitamin D, and of 'Nadola' (Parke Davis and Co.) which contains no vitamin D, is c. 100. A similar value was also obtained for vitamin A and carotene from cow's liver. It appears, therefore, that the utilization of carotene and vitamin A from these sources is complete, while that of carotene from plant material is less so and variable; the range of the biological values is 33-67. There is one exception in lettuce, the carotene of which is utilized as efficiently as is pure vitamin A.

What are the factors responsible for the variable utilization of carotene? The main factor is, no doubt, the composition of the carotene, i.e. the amount of β -carotene contained in the carotene mixture. Only few of the materials tested have as yet been thoroughly studied as to the forms of carotene they contain, e.g. carotene of leaf (Mackinney, 1935), spinach (Karrer & Schlientz, 1934; Beadle & Zscheile, 1942), carrots (Kuhn & Lederer, quoted by Brederick, 1936), apricots (Brockmann, 1933)

Table 1. *Biological values of carotenoids in various foodstuffs*

Biological value			
= $\frac{\text{Vitamin A potency of material in i.u.}}{\text{Carotene content of material in i.u.}} \times 100.$			
No. of test rats	Source	Kind of carotenoid	Biological value
10	'Nadola'*	Vitamin A	97
10	'Navitol'†	Vitamin A	100
10	Beef liver	" + carotene	100
10	"	" + carotene	101
10	Lettuce	Carotene	105
10	"	"	102
8	"	"	96
8	Maize leaves	"	67
8	"	"	66
10	Spinach	"	67
8	"	"	62
10	Carrots, raw	"	63
10	"	"	60
8	"	"	57
8	"	"	57
10	Carrots, cooked	"	62
8	"	"	54
10	Apricot	"	63
8	"	"	50
8	Dried grass	"	58
8	"	"	55
10	Tomato juice, fresh, concentrated	"	54
10	"	"	49
10	Tomato juice, canned, concentrated	"	42
10	"	"	39
10	"	"	37
10	"	"	34
10	Savoy cabbage	"	48
8	"	"	46
10	Carotene in olive oil†	"	46
10	"	"	40
8	"	"	47
8	"	"	43
10	Pumpkin	"	49
10	Maize flour	"	39
10	"	"	37
10	Alfalfa	"	39
10	"	"	33

* Vitamin A preparation (Parke Davis and Co.), contains 50,000 i.u. vitamin A/ml.

† Vitamin A preparation (E. R. Squibb and Sons), contains 50,000 i.u. vitamin A and 10,000 i.u. vitamin D/ml.

‡ Carotene (S.M.A. Corporation), contains 90% β -carotene and 10% α -carotene.

and maize (Sherman, 1940; Fraps & Kemmerer, 1941), but since the amount of pro-vitamin A found by chemical analysis is very much larger than the biological value, we must conclude, therefore, that, apart from the effect of the varying carotene mixture of different pro-vitamin A activity, there exists a factor (or factors), which influences the utilization of carotene by the organism.

Faecal excretion of carotene. One such factor may be faulty resorption from the intestine. Hence we studied carotene excretion in the faeces. Preliminary tests yielded the following results. (1) The

Table 2. *Excretion of carotene after feeding various amounts in foodstuffs*

No. of rats	Source of carotene	Amount of carotene given daily for 2 days (mg./rat)	Excretion of carotene	
			Total (mg.)	% of intake
5	Lettuce	0.078	0.106	68
8	"	0.033	0.044	66
5	Carrots	0.051	0.051	50
5	"	0.102	0.102	50
5	"	0.204	0.183	45
5	Maize flour	0.015	0.006	20
5	"	0.050	0.019	19
5	Carotene in olive oil	0.035	0.014	20
10.	"	0.050	0.019	19
8	"	0.080	0.028	18

faeces of rats not receiving carotene contained a pigment similar to carotene, which has already been described by several authors (Kemmerer & Fraps,

of carotene. There are, however, certain variations. We found, for instance, in 19 experiments on carrot carotene that the amount of carotene excreted varied between 39 and 60 % (average 49.9 %, S.D. 7.4 %); in 12 experiments with β -carotene in olive oil, 15–25 % was excreted (average 19.6 %, S.D. 4.4 %). A comparison of the amount excreted with the biological value of the carotene from the same material suggests that the biological value does not depend on the degree of excretion. On the contrary, the results show a correlation which appears paradoxical: a high biological value was found in cases of high faecal excretion and a low value in those of low excretion.

Effect of vitamin E on the utilization of vitamin A and carotene. Hickman, Harris & Woodside (1942) showed that vitamin E (α -tocopherol) improves the utilization of carotene and vitamin A in the curative growth test; they attribute this action of vitamin E to its anti-oxidative property, which protects vita-

Table 3. *Effect of α -tocopherol on the utilization and excretion of vitamin A and carotene*

No. of rats	Carotenoid given in 0.2 ml. olive oil on two successive days	α -tocopherol, amount given in 0.1 ml. olive oil together with the carotenoid (mg.)	Vitamin A found in liver (i.u./rat)	Biological value	Carotene excretion	
					Total (mg./rat)	% of intake
12	Vitamin A, 100 i.u.	0	18.5	100	—	—
8	"	0.1	22.0	122	—	—
8	"	0.5	25.0	144	—	—
8	"	2.0	31.0	196	—	—
8	"	10.0	45.0	c. 300	—	—
8	Carotene, 0.08 mg.	0	4.5	44	30	19
8	"	0.1	6.5	47	38	24
8	"	0.5	11.0	68	47	29
8	"	2.0	15.0	84	55	36
8	"	10.0	18.0	99	66	41

1938, Ramasarma & Hakim, 1942; Russel, Taylor, Walker & Polskin, 1942). The daily amount excreted in the faeces of the rat is equivalent to 0.5–2.0 μ g. carotene. (2) Carotene excretion by animals receiving carotene by mouth ceases one day after the last dose has been fed. On the following day the faeces are practically free from carotene.

In the experiments on carotene excretion the same procedure was used as in those on the biological value of carotene. On the first and second day of the experiment the rats received a measured amount of carotene; the faeces of these two days and of the following day were collected and their combined carotene content determined. For control purposes varying amounts of carotene from various sources were fed to different groups of rats.

From the results (Table 2) it would seem that (1) the percentage of carotene excreted is independent of the amount fed; (2) the amount of carotene excreted is more or less constant for each source

min A and carotene against oxidation. Their observations offer an explanation of our findings. (1) Protection against oxidation would account for the

Table 4. *Effect of α -tocopherol on the utilization and excretion of carotene from carrots*

The control rats received 10 i.u. vitamin A/10 g. body weight on two successive days. 6.0 i.u. vitamin A were recovered from each liver. The test rats received 1 g. carrots, containing 0.075 mg. carotene = 125 i.u. = 27.2 i.u./10 g. body weight, on two successive days.

No. of rats	α -tocopherol, amount given in 0.1 ml. olive oil together with carrots (mg.)	Vitamin A found in liver (i.u./rat)	Biological value	Carotene excretion	
				Total (mg./rat)	% of intake
8	0	13.2	57	68	45
8	0.05	19.8	81	81	54
8	0.2	20.2	83	83	55
8	0.5	23.7	102	86	57
8	1.0	27.2	119	104	69

good utilization of carotene as well as for the high excretion. (2) The highest biological value of plant carotene among the materials tested was that for lettuce carotene, and lettuce, as is well known, is characterized by its high vitamin E content.

To test the hypothesis regarding the effect of vitamin E both on the utilization of vitamin A and of carotene, and on carotene excretion, we carried out three sets of experiments in which experimental rats received measured amounts of vitamin A, or carotene in olive oil, or carrots, together with varying quantities of α -tocopherol. The amounts of vitamin A stored in the liver, and of carotene excreted, were examined. The experimental details and the results are given in Tables 3 and 4.

The data show that the addition of α -tocopherol to vitamin A, or carotene in olive oil, or carrots, raises the biological value of vitamin A and carotene and increases the amount of carotene excreted.

This dependence of the utilization of vitamin A and of carotene on dietary vitamin E possibly also explains the individual effect of various plant oils. To test this possibility we chose six oils: olive, sesame, coconut, cotton-seed, peanut and linseed; the olive oil was refined, the others unrefined. According to Karrer & Keller (1938), the respective amounts of α -tocopherol in olive, coconut, sesame and linseed oils are 0.08, 0.03, 0.05, 0.23 mg./g. According to Scudi & Buhs (1942), unrefined cotton-seed oil contains 0.92 mg. α -tocopherol/g.

DISCUSSION

The results of our estimations of the biological value of carotene and vitamin A agree more or less with those obtained by other authors using other methods. Smith & Otis (1941) observed that the amount of vitamin A found in the livers of avitaminotic rats fed on carotene varies according to the source of the carotene. Booher & Callison (1939) and Booher *et al.* (1939), using the dark adaptation test, studied the utilization of carotene by humans and found that 0.0006 mg. carotene in cotton-seed oil is equivalent to no more than 0.55–0.6 i.u. vitamin A and that the carotene of peas was better utilized than that of spinach. The results of our feeding experiments with various oils also agree with those of other authors. Sherman (1941) studied the effect of various oils on the carotene utilization in curative growth tests. He found that carotene in cotton-seed or linseed oil was better utilized than in coconut oil. The excretion of carotene was 11.4–16.7%. The results of Lease *et al.* (1939) differ somewhat from ours and from those of Sherman (1941), since they showed that carotene was better utilized when in coconut oil than in linseed oil. It is interesting to note that the refinement of peanut oil reduced its value as vehicle for carotene.

Davies & Moore (1941), the first to demonstrate a connexion in the metabolism of the vitamins A and E, found that addition of vitamin E to the

Table 5. Influence of various oils on the utilization and excretion of vitamin A and carotene

No. of rats	Carotenoid given in 0.5 ml. of the oil on two successive days	Oil	Vitamin A found in liver (i.u./rat)	Biological value	Carotene excretion	
					Total (mg./rat)	% of intake
8	Vitamin A, 100 i.u.	Olive, refined	13.0	100	—	—
8	"	Coconut, unrefined	15.0	112	—	—
8	"	Sesame, unrefined	13.0	100	—	—
8	"	Cotton-seed, unrefined	24.0	183	—	—
8	"	Peanut, unrefined	18.0	132	—	—
8	"	Linseed, unrefined	20.0	148	—	—
8	Carotene, 0.08 mg.	Olive, refined	4.3	44	27	17
8	"	Coconut, unrefined	4.5	45	32	20
8	"	Sesame, unrefined	4.2	44	39	24
8	"	Cotton-seed, unrefined	12.2	72	78	49
8	"	Peanut, unrefined	8.7	59	61	38
8	"	Linseed, unrefined	9.3	61	67	42

In our experiments we dissolved appropriate amounts of vitamin A or carotene in the various oils. These oils, containing 200 i.u. vitamin A or 0.16 mg. carotene/ml., were fed to the test rats. The experimental details and the results are given in Table 5. For the calculation of the biological values we took that of vitamin A in olive oil as 100. From the results (Table 5) it appears that the different effects of the various oils correspond roughly with their relative content of vitamin E.

diets of rats receiving vitamin A in cod-liver oil increased the amount of vitamin A stored in their livers. They also showed that the stores of vitamin A in the livers of rats on diets devoid of both vitamins A and E were more rapidly exhausted than those of rats receiving vitamin E. Bacharach (1940) confirmed these results. Hickman *et al.* (1942) demonstrated that vitamin E improved the vitamin A and carotene utilization in the curative growth test, and the latter authors showed, in *in vitro* experi-

ments, that vitamin E protected carotene against oxidation. These results, which we have confirmed, indicate the mechanism of the tocopherol effect: by preventing oxidation in the intestine, α -tocopherol increases both resorption and excretion of carotene.

We think that the utilization of carotene contained in plant materials depends above all on their vitamin E content. The case of lettuce is very significant in this connexion. Of the vegetables, it contains the highest amount of vitamin E, namely, 0.55 mg./g. of dried lettuce (Karrer & Keller, 1938), and, correspondingly, the biological value and the amount of lettuce carotene excreted exceed those of all other plant materials examined by us. The results of our experiments with various oils point in a similar direction (Table 5).

It appears, therefore, that in the rat vitamin E plays an important role in the metabolism of carotene and vitamin A by improving their utilization. There is no reason to doubt that vitamin E exerts a similar action on the utilization of carotene and vitamin A by humans. Vitamin E is, therefore, for humans not a 'vitamin', i.e. a nutritional factor essential for maintenance and growth, but rather one which is important for the utilization of other nutrients. This also follows from the studies of Pavcek & Shull (1942), who observed that the oxidation and inactivation of biotin by rancid fats is inhibited by α -tocopherol.

The utilization of vitamin A and carotene depends, therefore, chiefly on the amount of vitamin E contained in the food. This fact necessitates a new scrutiny of the problem of human vitamin A requirement. Our present assumptions concerning

this requirement do not take into account the vitamin E content of the food. The vitamin A requirement varies, probably, with the various vitamin E intakes, and a high amount of vitamin E in human diet will presumably decrease the vitamin A requirement.

SUMMARY

1. By the use of a liver storage test for the assessment of vitamin A, the utilization of vitamin A and of carotene from different sources was studied, and assessed in terms of biological values. The biological value of vitamin A contained in medical preparations and beef liver approaches 100; that of carotene in various plant materials ranges between 33 and 67, except in lettuce, in which it was found to be nearly 100.

2. The poor utilization of most of the plant carotenes is not caused by a large percentage of the less active or altogether inactive carotenoid pigments in these materials, or to a high carotene excretion with the faeces. The utilization of carotene from various plant sources, or of carotene dissolved in different oils, varies according to the vitamin E content of these materials.

3. Vitamin E increases both the utilization of vitamin A and of carotene, and the faecal excretion of carotene. Apparently it acts by protecting carotene and vitamin A against oxidation in the intestine, as a result of which their resorption and excretion are increased.

4. It is concluded that the human vitamin A requirement depends to a great extent on the vitamin E content of the food.

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