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SIXTY-SECOND SCIENTIFIC MEETING
DERBY HALL, LIVERPOOL

23 SEPTEMBER 1950

VITAMIN A

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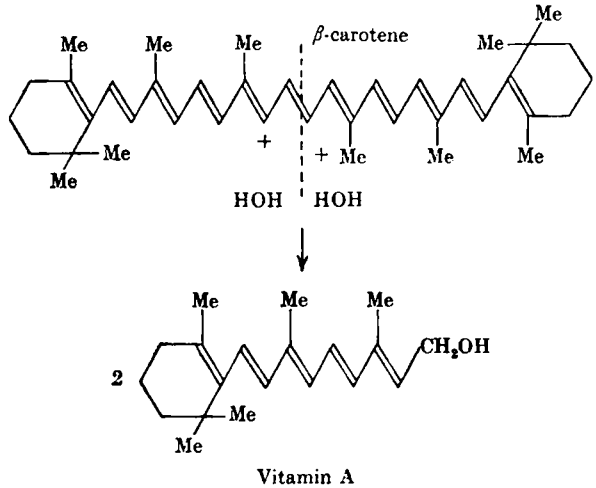
Vitamin A-Active Substances

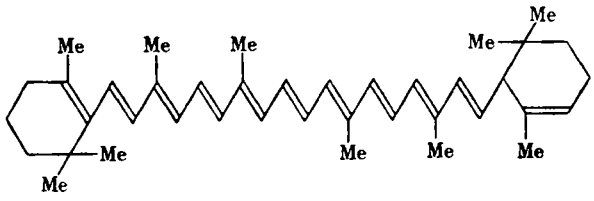
By T. W. Goodwin, *Department of Biochemistry, University of Liverpool*

Animals obtain their vitamin A either as such or as certain carotenoids which can be converted into vitamin A; such carotenoids are termed vitamin A precursors or provitamins A. For convenience, carotenoids and vitamin A and its derivatives can here be considered separately, but it should be emphasized that this differentiation is in a sense artificial because carotenoids are the ultimate source of all vitamin A. The carotenoid possessing the greatest vitamin A activity is β-carotene and the conversion can be represented qualitatively as a hydrolytic cleavage thus:

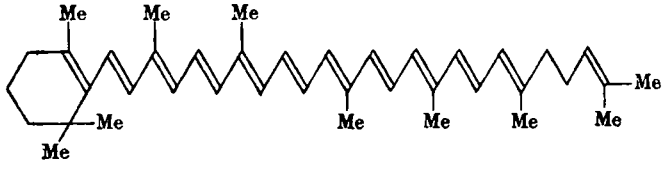


Vitamin A

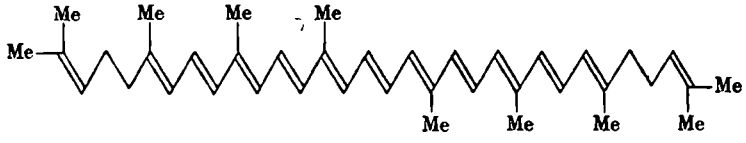
As α- and γ-carotenes are about one-half as active as β-carotene (Underhill & Coward, 1939; Kuhn & Brockmann, 1933; Wilkinson, 1941; von Euler, Karrer, Hellström & Rydbom, 1931) and lycopene is completely inactive (Karrer & Jucker, 1948), it follows that a β-ionone residue is a first essential for activity.



α-Carotene

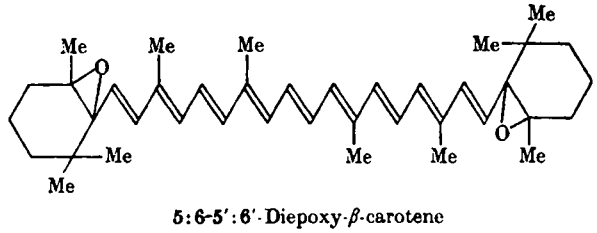


γ-Carotene



Lycopene

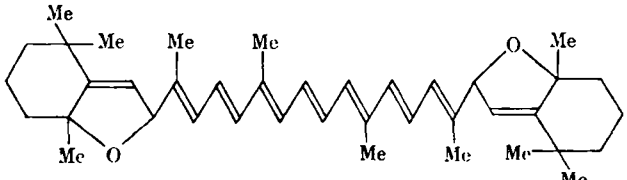
The complete inactivity of zeaxanthin (3:3′-dihydroxy-β-carotene) and of lutein (3:3′-dihydroxy-α-carotene) (von Euler, Karrer & Zubrys, 1934) and the relative activity of cryptoxanthin (3-hydroxy-β-carotene) which is about one-half that of β-carotene* (see Zechmeister, 1949) further indicates that the β-ionone residue must be unsubstituted; a very recent claim that astaxanthin (3:3′-dihydroxy-4:4′-diketo-β-carotene) is active in fish (Grangaud & Massonet, 1950) must, at the moment, be treated with great reservation. Recent work by Karrer and his associates suggests, however, that this criterion may have to be modified. Their recent work on the production of carotenoid epoxides indicates that 5:6-epoxides, e.g. 5:6-5′:6′-diepoxy-β-carotene, are vitamin A precursors (Karrer, Jucker, Rutschmann & Steinlin, 1945).



5:6-5′:6′-Diepoxy-β-carotene

It remains to be seen whether the criterion of an unsubstituted β-ionone residue will need to be discarded *in toto*, because it is quite possible that these epoxides are not active *per se* but by virtue of the fact that they are first converted into β-carotene. The furanoid (5:8-) epoxides, e.g. aurochrome (Karrer *et al.* 1945) are inactive.

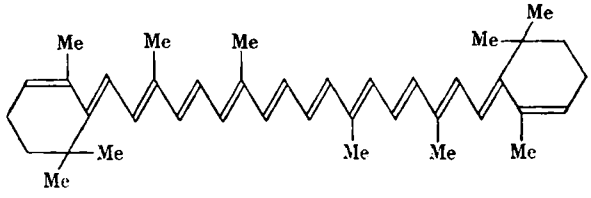
* If vitamin A storage instead of growth-promoting power is taken as the criterion of activity, cryptoxanthin appears to be almost as effective as β-carotene (Johnson & Baumann, 1948).



(Aurochrome) 5:8-5′:8′-diepoxy-β-carotene

The presence of a furanoid group does not, however, interfere with activity if the other end of the molecule contains an appropriate residue, e.g. mutatochrome (5:8-epoxy-β-carotene) and luteochrome (5:6-5′:8′-diepoxy-β-carotene) are active (Karrer & Rüegger, 1940; Gridgeman, Hunter & Williams, 1947).

The integrity of the isoprene side chain is a further essential requirement for full vitamin A activity, although it appears possible that some variations can be made without completely destroying activity (see Johnson, 1950). Two examples will clearly illustrate this. Hydrogenation of β-carotene with aluminium amalgam produces β-dihydrocarotene (7:8, 7′:8′-tetrahydro-β-carotene); this compound is without vitamin A activity (Karrer & Rüegger, 1940) as is the completely hydrogenated perhydro-β-carotene (von Euler, Demole, Karrer & Walker, 1930). Decomposition of the β-carotene-I_2 addition product with, for example, thiosulphate, yields *iso*carotene (dehydro-β-carotene) in which all activity is destroyed because a rearrangement of double bonds occurs, thus:



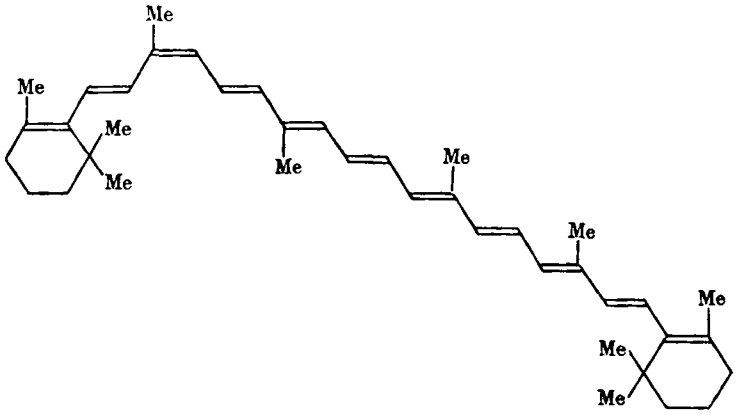
Dehydro-β-carotene

(Karrer & Schwab, 1940).

Unilateral oxidative degradation of the β-carotene molecule with the production of β-apocarotenes does not destroy activity so long as a vitamin A side chain remains present in the molecule, e.g. β-apo-8′-carotenal and β-apo-12′-carotenal are about as active as α-carotene (Karrer & Solmssen, 1937; Karrer, Rüegger & Geiger, 1938). α-Apocarotenes, on the other hand, e.g. α-apo-8-carotenal, are inactive because only an α-ionone residue remains (von Euler, Karrer & Solmssen, 1938).

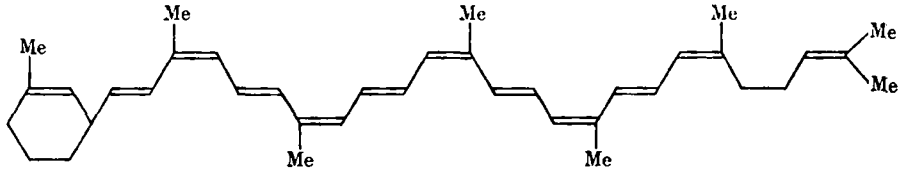
Carotenoids can exist in a number of stereoisomeric forms (see Zechmeister (1944) for a full discussion), but in general the naturally occurring form is the all-*trans* form. Under suitable conditions these all-*trans* forms can easily be converted into *cis*-isomers. Deuel and Zechmeister and their collaborators have, in a long series of papers (see Zechmeister (1949) and Goodwin (1951) for full references) measured the vitamin A activities of many of these *cis*-isomers, and have found that, with one important exception (see below), they are less active than the corresponding all-*trans* compounds. Some of these results have recently been independently confirmed by Bahl, Sadana & Ahmad (1948). A *trans* → *cis* rotation results in general in a carotenoid losing its

straight shape, e.g. neo-β-carotene U (3*-mono-*cis*-β-carotene), and Deuel and Zechmeister suggest that the lowered activity of *cis* carotenoids is due to the difficulty of the non-linear molecule fitting on to the 'carotenase' enzyme system.



Neo-β-carotene U

There remains the possibility, however, that neo-carotenoids are not active *per se* but are first re-arranged in the intestinal tract to β-carotene; in fact, Kemmerer & Fraps (1945) state that they have obtained evidence of such a re-arrangement. Deuel's and Zechmeister's objection to this suggestion is based on their experience with pro-γ-carotene. This carotenoid is a naturally occurring poly-*cis*-isomer (Zechmeister terms such naturally occurring *cis*-compounds 'pro-carotenoids'), probably 3:5:7:9:11-penta *cis*-γ-carotene, which has a biological activity indistinguishable from that of the all-*trans*-γ-carotene. In pro-γ-carotene all possible *trans* → *cis* rotations have occurred, for rotation of all the double bonds cannot take place owing to steric hindrance (Zechmeister, 1944), and the molecule is no longer bent:



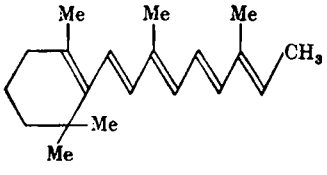
Pro-γ-carotene

This molecule, it is assumed, can fit on to the appropriate enzyme as easily as all-*trans*-γ-carotene and thus the two compounds have the same vitamin A activity.

Vitamin A is a primary alcohol, and it is interesting to observe the effect on the biological activity of the molecule of altering the terminal hydroxyl grouping. Vitamin A esters are of course active, being hydrolysed in the gut, absorbed in the free state and re-esterified during the passage across the gut wall (Gray, Morgareidge & Cawley,

* In Zechmeister's nomenclature italicized numerals refer to the double bonds in the molecule and not to the carbon atoms.

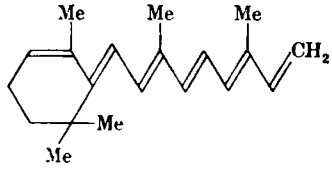
1940). Retinene (vitamin A aldehyde) is active (Glover, Goodwin & Morton, 1948*a*) and so are the ethers derived from vitamin A and its higher homologues (see Embree (1947) and Milas (1947) for full references), vitamin A acid (Arens & van Dorp, 1946; Sharman, 1949) and dimethylaminovitamin A (Milas, 1947). Karrer & Benz(1948) prepared the hydrocarbon corresponding to vitamin A (axerophthene) and found that it was biologically active; they concluded that the substituents on the terminal carbon atoms were of little significance in controlling activity. Taken with the results just quoted this appears to be valid, but it should be noted, however, that Karrer, Patel & Benz (1949) have recently reported that 15-ethylaxerophthene is inactive, and that Meunier and his collaborators (Meunier, 1948; Guerillot-Vinet, Meunier, Jouanneteau & Gourevitch, 1948) deny biological activity to axerophthene. Any shortening of the side chain of vitamin A completely destroys biological activity (Karrer & Benz, 1948), but apparently some variations can be made on the side chain without eliminating activity completely. As full details of compounds of this type are not always available, they will not be discussed further. A recent review of the organic chemistry of these compounds which has recently appeared (Johnson, 1950) also included some data on their biological activity.



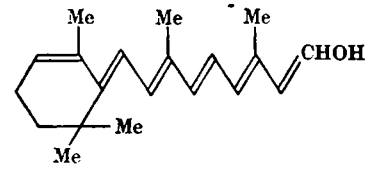
Axerophthene

Vitamin A, according to Zechmeister (1944), can exist in four possible stereoisomeric forms because bonds *3* and *5* are sterically unhindered. Leaving aside the possibility of the compounds not being completely pure, the varying activities reported for synthetic vitamin A derivatives synthesized by different routes may be due to the production of *cis*-isomers (see Milas, 1947; Embree, 1947). Only one vitamin A isomer has as yet been unequivocally identified; Robeson & Baxter (1947) isolated *neo*-vitamin A and consider it to be 5-*cis*-vitamin A. Rather surprisingly, when the activity of *cis*-carotenoids is remembered, it has the same biological potency as vitamin A itself.

The position of anhydrovitamin A is interesting since Shantz (1950) has emphasized that it has a small but significant potency (0·4 % that of vitamin A); as it possesses the same disposition of double bonds as dehydro-β-carotene, this very low value is not



Anhydrovitamin A



Rehydrovitamin A

surprising since the carotenoid is apparently inactive. Anhydrovitamin A is, however, stored in the liver as a compound containing an hydroxyl group; this compound, termed rehydrovitamin A by Shantz, when fed to rats is twenty times as active as anhydrovitamin A. Vitamin A can form a 5:6-epoxide (Karrer & Jucker, 1945) which,

however, appears to have very little activity. Kitol ($C_{40}H_{58}(OH)_2$) which occurs in mammalian liver oils, being especially abundant in whale-liver oils, is apparently a dimer of vitamin A (see Harris, 1949; Barua & Morton, 1949) and has no biological activity *per se*, but yields vitamin A on heat treatment.

A derivative of vitamin A that has recently aroused much interest is vitamin A_2; although widely distributed in both marine and freshwater fish this compound is relatively much more abundant in the latter. It is active *per se* in the rat, replacing vitamin A completely without in any way being converted into it (Shantz, Embree, Hodge & Willis, 1946). Its activity is less than that of vitamin A, Shantz & Brinkman (1950) reporting a potency of $1{\cdot}3 \times 10^6$ i.u./g. Evidence is now accumulating that vitamin A_2 is 3-dehydrovitamin A (Morton, Salah & Stubbs, 1947; Morton, Cama, Dalvi, Field & Salah, 1950). If this is so, it means that animals can utilize a dehydro-β-ionone residue; unfortunately no naturally occurring carotenoid is yet known which contains such a residue, and thus a direct demonstration of a conversion of such a compound into vitamin A_2 is not, at the moment, possible. On the other hand, freshwater fish apparently have the ability to dehydrogenate β-carotene in this way, since Morton & Creed (1939) have demonstrated the conversion of β-carotene into vitamin A_2 in dace and perch.

One of the most important recent observations in the study of vitamin A-active substances is undoubtedly that vitamin A acid is potent without apparently being converted into vitamin A (Arens & van Dorp, 1946; Glover, Goodwin & Morton, 1948*b*; Sharman, 1949).

Apart from its function in vision, the major physiological activity of vitamin A is in preserving the integrity of the epithelial tissues but, in spite of this, the presence of vitamin A has never been unequivocally demonstrated at this site of action. The observations on vitamin A acid strongly suggest that we may have been premature in accepting the suggestion that the alcohol is the active form of the vitamin; this may well be only an intermediate in the conversion of the stored vitamin esters into the, as yet unrecognized, active principle.

REFERENCES

Arens, J. F. & van Dorp, D. A. (1946). *Nature, Lond.*, **157**, 190.
Bahl, A. N., Sadana, J. C. & Ahmad, B. (1948). *J. sci. industr. Res., India*, **7**B, 169.
Barua, R. K. & Morton, R. A. (1949). *Biochem. J.* **45**, 308.
Embree, N. D. (1947). *Ann. Rev. Biochem.* **16**, 323.
Glover, J., Goodwin, T. W. & Morton, R. A. (1948*a*). *Biochem. J.* **43**, 109.
Glover, J., Goodwin, T. W. & Morton, R. A. (1948*b*). Unpublished work.
Goodwin, T. W. (1951). *The Biochemistry of the Carotenoids.* London: Chapman and Hall. (In the Press.)
Grangaud, R. & Massonet, R. (1950). *C.R. Acad. Sci., Paris*, **230**, 1319.
Gray, E. le B., Morgareidge, K. & Cawley, J. D. (1940). *J. Nutrit.* **20**, 67.
Gridgeman, N. T., Hunter, R. F. & Williams, N. E. (1947). *J. chem. Soc.* p. 131.
Guerillot-Vinet, A., Meunier, P., Jouanneteau, J. & Gourevitch, M. (1948). *C.R. Acad. Sci., Paris*, **226**, 128.
Harris, P. L. (1949). *Ann. Rev. Biochem.* **18**, 391.
Johnson, A. W. (1950). *Rep. Progr. Chem.* **46**, 168.
Johnson, R. M. & Baumann, C. A. (1948). *Arch. Biochem.* **19**, 493.
Karrer, P. & Benz, J. (1948). *Helv. chim. Acta*, **31**, 1048.
Karrer, P. & Jucker, E. (1945). *Helv. chim. Acta*, **28**, 717.

Karrer, P. & Jucker, E. (1948). *Carotinoide.* Basel: Birkhauser.
Karrer, P., Jucker, E., Rutschmann, J. & Steinlin, K. (1945). *Helv. chim. Acta*, **28**, 1146.
Karrer, P., Patel, D. K. & Benz, J. (1949). *Helv. chim. Acta*, **32**, 1938.
Karrer, P. & Rüegger, A. (1940). *Helv. chim. Acta*, **23**, 955.
Karrer, P., Rüegger, A. & Geiger, A. (1938). *Helv. chim. Acta*, **21**, 1171.
Karrer, P. & Schwab, G. (1940). *Helv. chim. Acta*, **23**, 578.
Karrer, P. & Solmssen, U. (1937). *Helv. chim. Acta*, **20**, 682.
Kemmerer, A. R. & Fraps, G. S. (1945). *J. biol. Chem.* **161**, 305.
Kuhn, R. & Brockmann, H. (1933). *Ber. dtsch. chem. Ges.* **66**, 1319.
Meunier, P. (1948). *C.R. Acad. Sci., Paris*, **227**, 206.
Milas, N. A. (1947). *Vitamins and Hormones*, **5**, 1.
Morton, R. A., Cama, H. R., Dalvi, P. D., Field, A. C. & Salah, M. K. (1950). Unpublished.
Morton, R. A. & Creed, R. H. (1939). *Biochem. J.* **33**, 318.
Morton, R. A., Salah, M. K. & Stubbs, A. L. (1947). *Nature, Lond.*, **159**, 744.
Robeson, C. D. & Baxter, J. G. (1947). *J. Amer. chem. Soc.* **69**, 136.
Shantz, E. M. (1950). *J. biol. Chem.* **182**, 515.
Shantz, E. M. & Brinkman, J. H. (1950). *J. biol. Chem.* **183**, 467.
Shantz, E. M., Embree, N. D., Hodge, H. C. & Willis, J. H. (1946). *J. biol. Chem.* **163**, 455.
Sharman, I. M. (1949). *Brit. J. Nutrit.* **3**, viii.
Underhill, S. W. F. & Coward, K. H. (1939). *Biochem. J.* **33**, 589.
von Euler, H. Demole, V., Karrer, P. & Walker, O. (1930). *Helv. chim. Acta*, **13**, 1078.
von Euler, H., Karrer, P., Hellström, H. & Rydbom, M. (1931). *Helv. chim. Acta*, **14**, 839.
von Euler, H., Karrer, P. & Solmssen, U. (1938). *Helv. chim. Acta*, **21**, 211.
von Euler, H., Karrer, P. & Zubrys, A. (1934). *Helv. chim. Acta*, **17**, 24.
Wilkinson, H. (1941). *Biochem. J.* **35**, 824.
Zechmeister, L. (1944). *Chem. Rev.* **34**, 267.
Zechmeister, L. (1949). *Vitamins and Hormones*, **7**, 59.

Vitamins A and Vision

By R. A. MORTON, *Department of Biochemistry, University of Liverpool*

According to the ideas now current, the following sequence obtains in the formation of visual purple: (1) The animal ingests carotenoid provitamins, and sometimes preformed vitamin A, but absorption of unchanged carotenoids is small or very small indeed, depending on the species. (2) An enzyme system (carotenase?) operating in the gut wall results in fission of the C_{40} molecules to C_{20} molecules and vitamin A aldehyde is formed, to be rapidly reduced to free vitamin A. (3) Much of the vitamin A is esterified in the gut wall and then transported to the liver to be stored largely as ester in the Kupffer cells. Either in the true liver cells, or in the blood plasma, or in both, an esterase acts on the vitamin, and the outcome is a fairly constant plasma level of the order of 1 i.u. (0·3 μg.) free vitamin A per ml. (4) A small but fairly constant amount of vitamin A is found in the pigment epithelium readily accessible to the retina. (5) In the process of dark adaptation the reddish pigment, rhodopsin (visual purple), accumulates in the rods of the retina, but the process is delayed when the blood level of vitamin A is low; there is then defective scotopic vision, that is to say vision in light of low intensity. (6) Exposure to bright light results in decomposition of rhodopsin in vivo but the pigment is regenerated in the dark. (7) In some freshwater fishes and amphibia rhodopsin is replaced by porphyropsin and vitamin A_1 by vitamin A_2. The latter differs from the former in having one additional conjugated double bond in the substituted six-membered ring. (8) Photopic vision, which is vision in light of high