P AUL K ARRER

Carotenoids, flavins and vitamin A and B$_2$

Nobel Lecture, December 11, 1937

In the last few decades chemical research has been divided in the main into two directions; one part aims at the investigation of the structure of the atom in the widest sense, the other at the elucidation of the composition of the living cell and its chemical reactions. The first direction has availed itself in many ways of entirely new methods of investigation, which are essentially of a physical nature. Biochemical research was able to base itself so far in the main on the methods of classical chemistry, which certainly have been improved and developed in an extraordinary manner.

Progress in methods which modern biochemistry can no longer dream of doing without, are the selective adsorption processes of Willstätter, separation by the ultracentrifuge of Svedberg, and chromatographic analysis by Tsvett, by the use of which it is now possible to separate mixtures of substances which were previously inextricable. Chromatographic analysis, in particular, which is based upon filtration of the solution of the mixture of substances through an adsorption column, in which the more easily adsorbed substances are retained in the upper part of the tube, with the substances more difficult to adsorb being retained in the lower part, operates in a similarly complete manner like a prism, which divides the white light into the individual colours of the spectrum. Thanks to these efficient separation processes our picture of the composition of plant and animal cells is today essentially more complicated than it was a few years ago, and it cannot be doubted that to later generations there will be revealed an even greater diversity and non-uniformity of the living cell.

The blue and red pigments of blossoms and berries, the anthocyanins, which were formerly thought to be uniform and characteristic for the various plants concerned, have been shown by later investigations to be mixtures of many closely related substances. There is indeed not one flower, not one berry, which owes its colouration to a single anthocyanin. Similar conditions apply in another group of natural pigments, the carotenoids, about which I have the honour to speak today. In the well-known book by L. S. Palmer on carotenoids which was published in 1922, only six carotenoids are

described which had been crystallized and analysed (carotene, lycopene, xan-thophyll, lutein, fucoxanthin, and rhodoxanthin). Bixin was also known at that time. Until four years ago the number of these naturally occurring pigments had risen to 15 ; today we know already some 40 natural pigments of this group, the isolation of which has only been made possible by the new methods of separation.
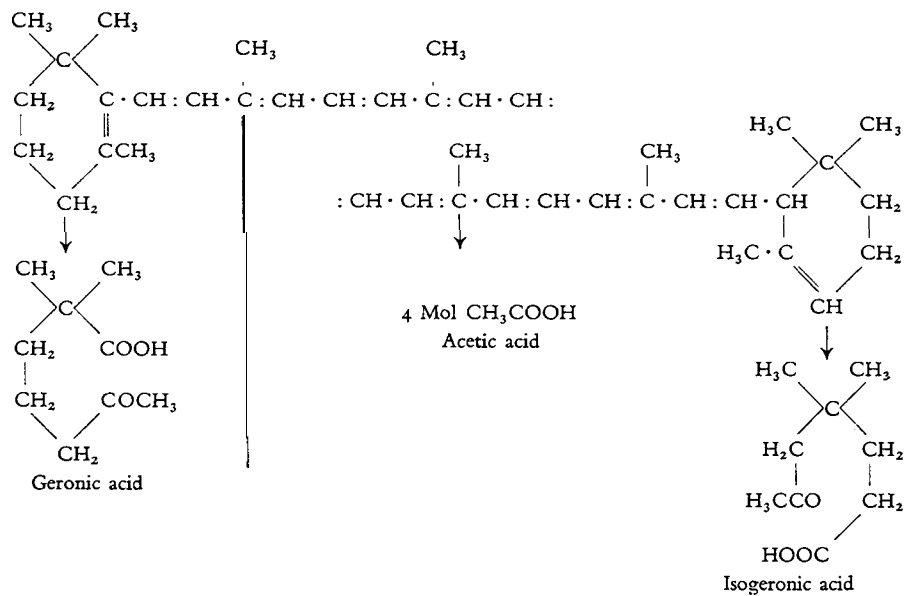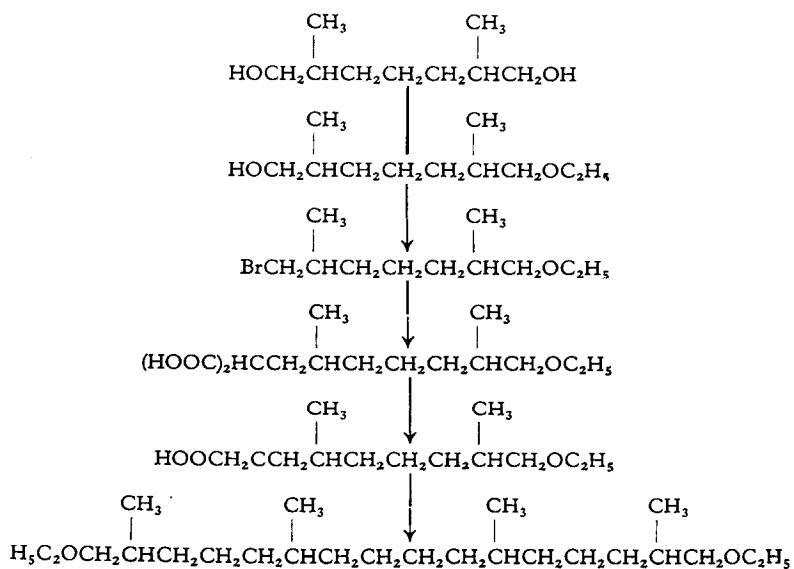
Attention has been paid to the carotenoids in more recent research, not only because of their unique structure, but also because of their close relation to the vitamins, as some carotenoids are provitamins of vitamin A and are transformed into this vitamin in the animal organism. Investigation of the structural constitution of the vitamins started from carotene; when it was possible, in 1930, to establish the correct constitutional formula for b-caro-tene, a structural formula for any other vitamin or provitamin was not yet known. Shortly afterwards, in 1931, it became also possible to elucidate the constitution of vitamin A itself, and thus to obtain the first insight into the structure of a vitamin. We may perhaps remember that scarcely ten years have elapsed since the time when many research scientists doubted the mate-rial specificity of the vitamins, and were of the opinion that a special state of matter, a special colloidal character, was the cause of the peculiar vitamin effects which had been observed.

Carotene and its relatives are polyenes; they contain numerous conjugated double bonds in their molecules. We discovered this characteristic structural principle for the first time when investigating the saffron pigment, crocetin. As examples of the molecular structures of such polyenes I give here the formulae of crocetin, bixin (norbixin), the tomato pigment lycopin and fi-carotene (see opposite page).

To the constitutionally clarified carotenoids there also belong α- and γ-carotenes, the second yellow Pigment xanthophyll, whose wide distribution in the animal kingdom has been demonstrated by Lönnberg in particular, and also zeaxanthin from maize and many other fruits, the pigment of lob-sters and many other crustacea, astacin, the paprika pigment capsanthin, cryptoxanthin, and many others.

What is striking in the above structural formulae is not only the abundance of conjugated double bonds, unknown previously in any other natural sub-stances, but the fact that the carotenoid molecules are composed of isoprene residues. This applies for all carotenoids which are therefore to be recognized as relatives of the terpenes, camphor, and rubbers. Furthermore, the sym-metrical character of the above four formulae is striking; they are composed

$$\text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3$$
$$\text{HOOC} \cdot \text{C} : \text{CH} \cdot \text{CH} : \text{CH} \cdot \text{C} : \text{CH} \cdot \text{CH} : \text{CH} \cdot \text{CH} : \text{C} \cdot \text{CH} : \text{CH} \cdot \text{CH} : \text{C} \cdot \text{COOH}$$

Crocetin

$$\text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3$$
$$\text{HOOCCH} : \text{CH} \cdot \text{C} : \text{CH} \cdot \text{CH} : \text{CH} \cdot \text{C} : \text{CH} \cdot \text{CH} : \text{CH} \cdot \text{CH} : \text{C} \cdot \text{CH} : \text{CH} \cdot \text{CH} : \text{C} \cdot \text{CH} : \text{CHCOOH}$$
[CH$_3$]   Nor-bixin Bixin [CH$_3$]

$$\text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3$$
$$(\text{CH}_3)_2\text{C} : \text{CHCH}_2\text{CH}_2\text{C} : \text{CH} \cdot \text{CH} : \text{CH} \cdot \text{C} : \text{CH} \cdot \text{CH} : \text{CH} \cdot \text{C} : \text{CH} \cdot \text{CH} :$$
$$\text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3$$
$$: \text{CH} \cdot \text{CH} : \text{C} \cdot \text{CH} : \text{CH} \cdot \text{CH} : \text{C} \cdot \text{CH} \cdot \text{CH} : \text{C} \cdot \text{CH}_2\text{CH}_2\text{CH} : \text{C}(\text{CH}_3)_2$$

Lycopin

β-Carotene

of two equal halves, and so are centro-symmetrical – both ends of the molecule show the same structure. This structural principle, which we first recognized in the tomato pigment lycopin and then in carotene, later proved to be realized in other branches of terpene chemistry and was applied by various research workers with success in the derivation of structural formulae.

The structural formulae of the above-mentioned four carotenoids have been proved partly by decomposition reactions and partly by synthesis of their perhydroderivatives.

Two examples of this type are shown in the following structural formulae, one showing constitutional determination by decomposition and the other by synthesis.

Oxidative decomposition of a-carotene

CH$_3$ CH$_3$
     \ /
      C
     / \
CH$_2$ C · CH : CH · C : CH · CH : CH · C : CH · CH :
| ‖
CH$_2$ CCH$_3$
     \ /
     CH$_2$
      ↓
CH$_3$ CH$_3$
     \ /
      C
     / \
CH$_2$ COOH
|
CH$_2$ COCH$_3$
     \ /
     CH$_2$
Geronic acid

CH$_3$ CH$_3$

: CH · CH : C · CH : CH · CH : C · CH : CH · CH

CH$_3$ CH$_3$

          ↓

    4 Mol CH$_3$COOH
        Acetic acid

H$_3$C   CH$_3$
     \ /
      C
     / \
H$_3$C · C   CH$_2$
| |
           CH$_2$
      \\ /
       CH
        ↓
H$_3$C   CH$_3$
     \ /
      C
     / \
H$_2$C   CH$_2$
| |
H$_3$CCO   CH$_2$
       \ /
HOOC
Isogeronic acid

Synthesis of perhydrocrocetin

CH$_3$ CH$_3$
 | |
HOCH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$OH

CH$_3$ CH$_3$
 | |
HOCH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$OC$_2$H$_5$

CH$_3$ CH$_3$
 | |
BrCH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$OC$_2$H$_5$

CH$_3$ CH$_3$
 | |
(HOOC)$_2$HCCH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$OC$_2$H$_5$

CH$_3$ CH$_3$
 | |
HOOCH$_2$CCH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$OC$_2$H$_5$

CH$_3$ CH$_3$ CH$_3$ CH$_3$
 | | | |
H$_5$C$_2$OCH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$CH$_2$CH$_2$CH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$OC$_2$H$_5$

$$\overset{\text{CH}_3}{|} \qquad \overset{\text{CH}_3}{|} \qquad \downarrow \qquad \overset{\text{CH}_3}{|} \qquad \overset{\text{CH}_3}{|}$$

HOCH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$OH

$$\overset{\text{CH}_3}{|} \qquad \overset{\text{CH}_3}{|} \qquad \downarrow \qquad \overset{\text{CH}_3}{|} \qquad \overset{\text{CH}_3}{|}$$

HOOCCHCH$_2$CH$_2$CH$_2$CHCH$_2$CH$_2$CH$_2$CHCH$_2$CH$_2$CH$_2$CHCOOH

Perhydrocrocetin

However, the theory now allows us to foresee furthermore for each individual carotenoid extraordinarily numerous stereoisomeric forms, since at each individual double bond the substituents can be arranged either in *cis*-position or *trans*-position, whereby even two modifications of the *trans*-arrangement must be taken into consideration. Diagrammatically the three extreme cases (continuous *cis*-form, continuous attenuated *trans*-form, and continuous compressed *trans*-form) can be represented as follows:

cis-form   attenuated *trans*-form   compressed *trans*-form

Theoretically a very large number of stereoisomers can be imagined by combining *cis*- and *trans*-arrangements. As in many other cases, however, Nature has made only slight use of these possibilities. Up to the present we know only two carotenoid pigments in which this *cis-trans*-isomerism is realized. These are bixin and crocetin, which occur in both forms as unstable and stable isomers.

Despite the wealth of possibilities of isomerism which are available here, it can be established with great certainty, in the case of the unstable and stable bixin, which double bond is responsible for the occurrence of the two isomers. It is possible to decompose the polyenes by permanganate oxidation in stages to aldehydes in the sense that one double bond after another is oxidized. In this way the aldehydes I to III were obtained from unstable bixin:

$$\overset{\text{CH}_3}{|} \qquad\qquad \overset{\text{CH}_3}{|}$$

CH$_3$OOC · CH ∶ CH · C ∶ CH · CH ∶ CH · C ∶ CH · CH ∶

$$\qquad\qquad\qquad\qquad \overset{\text{CH}_3}{} \qquad\qquad\qquad \overset{\text{CH}_3}{}$$

∶ CH · CH ∶ C · CH ∶ CH · CH ∶ C · CH ∶ CH · COOH

Bixin

$$\left.\begin{array}{l} \overset{\displaystyle CH_3}{\displaystyle |} \qquad\qquad \overset{\displaystyle CH_3}{\displaystyle |} \\ CH_3OOC \cdot CH : CH \cdot C : CH \cdot CH : CH \cdot C : CH \cdot CH : \\ \qquad\qquad\qquad\qquad\qquad \overset{\displaystyle CH_3}{\displaystyle |} \qquad\qquad \overset{\displaystyle CH_3}{\displaystyle |} \\ \qquad\qquad\qquad\qquad\quad : CH \cdot CH : C \cdot CH : CH \cdot CH : C \cdot CHO \end{array}\right\} \quad (I)$$

$$\overset{\displaystyle CH_3}{\displaystyle |}\qquad\quad \overset{\displaystyle CH_3}{\displaystyle |}\qquad\quad \overset{\displaystyle CH_3}{\displaystyle |}$$
$$CH_3OOC \cdot CH : CH \cdot C : CH \cdot CH : CH \cdot C : CH \cdot CH : CH \cdot CH : C \cdot CH : CHCHO \quad (II)$$

$$\overset{\displaystyle CH_3}{\displaystyle |}\qquad\quad \overset{\displaystyle CH_3}{\displaystyle |}\qquad\quad \overset{\displaystyle CH_3}{\displaystyle |}$$
$$CH_3OOC \cdot CH : CH \cdot C : CH \cdot CH : CH \cdot C : CH \cdot CH : CH \cdot CH : C \cdot CHO \quad (III)$$

In the same way decomposition of stable bixin yields aldehydes of structures I to III. Whereas the compounds III are identical in both cases, the aldehydes I and II obtained from unstable and stable bixin proved to be different; they are *cis-trans*-isomers. From this it can be seen that the stereoisomerism of the two natural bixins is very probably based on the different configuration of the third double bond, counted from the free carboxyl.* If a *trans*-form is assumed for the remaining double bonds (which for various reasons is not improbable, though not proved), then the differences in structure of the two isomeric bixins can be expressed by the following formulae:

Unstable bixin

Stable bixin

* An objection to this conclusion could be brought forward, viz. that a labile apo-3-norbixinal methyl ester (Formula III), in contrast to the labile apo-1-norbixinal methyl ester and apo-2-norbixinal methyl ester (Formulae I and II), could rearrange *spontaneously* to a stable form under the conditions of the oxidation.

Even fine stereochemical details of such complex structural molecules are thus experimentally accessible to test and clarification.

Reference to the close relationship of β-carotene to vitamin A has already been made. H. v. Euler recognized that carotene can replace vitamin A in experiments on animals, and Th. Moore proved that in the animal organism it is transformed into vitamin A. These near relationships became understandable when we succeeded in isolating and constitutionally elucidating vitamin A. The structural formula, which is proved by a synthesis of the perhydro-vitamin A, shows that the compound contains half of the carbon skeleton of β-carotene and from this, by splitting at the central double bond, the result must have been:

$$\text{CH}_3 \quad \text{CH}_3$$
$$\text{C}$$
$$\text{CH}_2 \quad \text{C}\cdot\text{CH}:\text{CH}\cdot\text{C}:\text{CH}\cdot\text{CH}:\text{CH}\cdot\text{C}:\text{CH}\cdot\text{CH}_2\text{OH}$$
$$\text{CH}_2 \quad \text{C}\cdot\text{CH}_3$$
$$\text{CH}_2$$
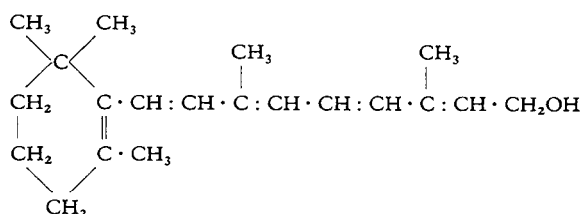
Recently this compound was given the name of axerophtol.

It appeared now to be of interest to establish whether still further carotenoids, besides β-carotene, could be decomposed through the animal organism, presumably through its liver tissue, to axerophtol. Investigation of this problem, which was carried out together with H. v. Euler, led first of all to the recognition that this does not hold true for most other natural carotenoids. Substitution by OH or keto groups, splitting of the carbon ring of β-carotene, etc., leads to a complete disappearance of the vitamin A effect. Besides β-carotene, in whose molecule the unsubstituted carbon skeleton of vitamin A occurs twice, those carotenoids proved still to be effective which contain the carbon skeleton in the unsubstituted form at least once. Pigments of this kind are α-carotene, γ-carotene*, cryptoxanthin*, echinenon**, a β-carotene oxide, dihydro-β-carotene, dihydro-α-carotene, diiodo-β-carotene, β-semicarotinone* and also β-apo-2-carotinal and β-apo-4-carotinal. The structural formulae of some compounds of this provitamin group show their close constitutional relationships to vitamin A:

 * R. Kuhn *et al.*
** E. Lederer

CH₃   CH₃

$$\text{CH}_2 \quad \text{C—CH=CH—C=CH—CH=CH—C=CH—CH=}$$

with CH₃ above at positions, CH₂ below

(α-Carotene structure)

$$=\text{CH—CH=C—CH=CH—CH=C—CH=CH—CH} \qquad \text{CH}_2 \qquad \text{α-Carotene}$$

CH₃ ... CH₃ ... H₃C ... CH₂ ... H₃CC ... CH₂ ... CH

$$----\ =\text{CH—CH=C—CH=CH—CH=C—CH=CH—CH=C—CH}_2\text{CH}_2\text{CH=C}\begin{smallmatrix}\text{CH}_3\\\text{CH}_3\end{smallmatrix} \qquad \text{γ-Carotene}$$

$$\cdots\cdots\ =\text{CH—CH=C—CH=CH—CH=C—CH=CH—C} \qquad \text{CH}_2 \qquad \text{Cryptoxanthin}$$

H₃C, CH₃ on C; H₃CC, CHOH, CH₂

$$----\ =\text{CH—CH=C—CH=CH—CH=C—CH=CH—CO} \qquad \text{CH}_2 \qquad \text{β-Semicarotenone}$$

H₃C, CH₃ on C; H₃CCO, CH₂, CH₂

$$----\ =\text{CH—CH=C—CH=CH—CH=CCHO} \qquad \text{β-Apo-2-carotenal}$$

with CH₃, CH₃ above

$$----\ =\text{CH—CH=CCHO} \qquad \text{β-Apo-4-carotenal}$$

with CH₃ above

The last three of these provitamins are synthetic products, which have been formed by oxidative splitting at the first, second, or fourth double bond of the carotene molecule.

If a single double bond has changed its location in the carbon skeleton

characteristic for vitamin A, it is sufficient to neutralize its action as provitamin A. In this way α-semicarotenone, which differs from effective *β-semi-*carotenone by displacement of the double bond in the carbon ring, proved to be quite ineffective:

α-Semicarotenone

β-Semicarotenone
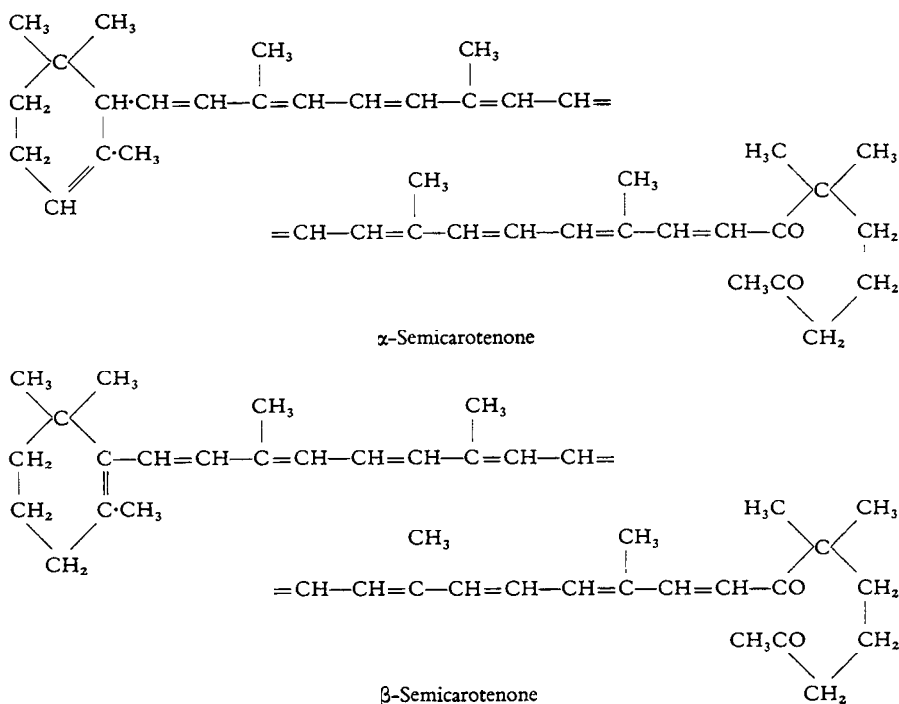
The action of vitamin A is consequently extraordinarily specific and bound to a quite definite structure. Today it is possible to show the atom arrangement in a polyene characteristic of vitamin A more reliably by experiment on animals than by any chemical decomposition reaction or by spectrum analysis measurements. This high specificity is all the more surprising if it is remembered that with regard to most hormones, e.g. the female sexual hormones and the plant growth hormones, we can hardly speak of a constitutional specificity. Not only do substances which are chemically closely related produce there similar physiological results, but it can often happen that compounds of entirely different constitutions show the same effects as the. natural products.

This strongly pronounced constitutional specificity of vitamin effects may be connected with the results of the most recent investigations, which re-

vealed that many vitamins are functional groups of enzymes, the highly specific properties of which have long been known. These close relationships between enzymes and vitamins were first shown in vitamin B_2 or lactoflavin. According to the investigations of Warburg and Theorell the phosphoric acid ester of vitamin B_2 is the functional group of the yellow oxidation enzyme. Today lactoflavin and any other flavin can be obtained synthetically, and hence we were able to investigate at this point to what extent the vitamin effect is bound to a certain constitution.

The process of such a flavin synthesis, one giving the best yields, may be sketched very briefly. Aromatic primary amines are reductively condensed with sugars. These result in compounds of type (a). They can then be coupled with diazonium salts to azo-pigments (b). By reduction, diaminoderivatives of formula (c) are obtained, which combine with alloxan to form the flavin pigment.
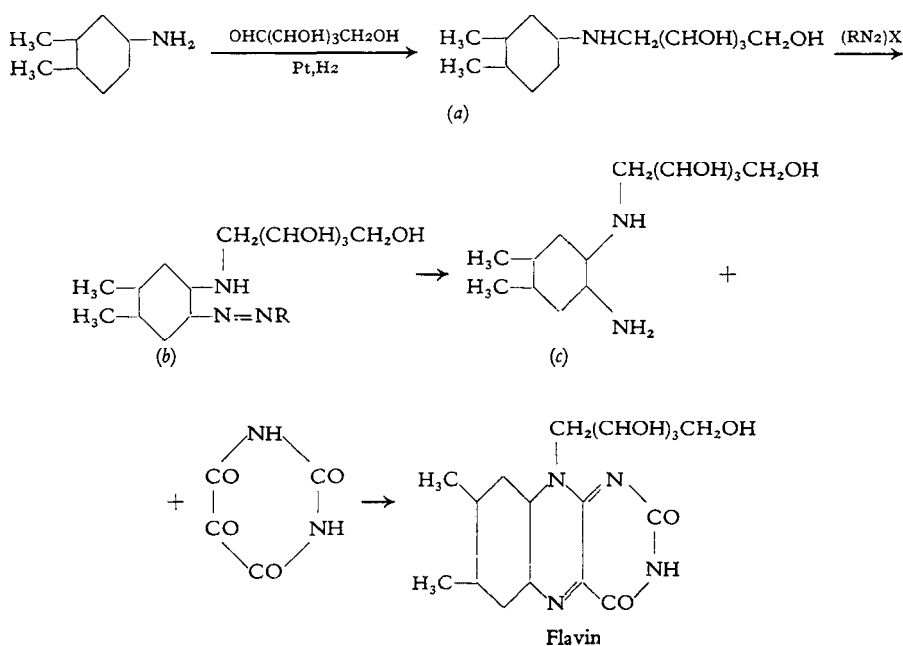
By using this and similar methods more than 25 different flavins have been obtained in our laboratory, which differ from one another by the nature of the sugar residues or the substitution in the aromatic ring, amongst them being lactoflavin itself. This experimental material formed the basis

for an assessment of the constitutional specificity of the action of vitamin B_2. As with vitamin A, with lactoflavin the amplitude of variation is extremely narrow. A substitution of the *d*-ribose residue in the lactoflavin

$$CH_2CHOHCHOHCHOHCH_2OH \quad (\textit{d–Ribose rest})$$
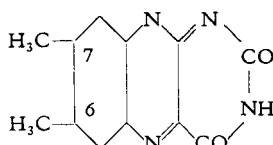
by other sugars practically abolishes the action; only the arabinose compounds still show a weak, stimulating effect. Just as much significance have the two methyl groups in positions 6 and 7. One or the other can indeed still be omitted or substituted by ethyl without the vitamin action being substantially affected. On the other hand, the disappearance of both methyl residues results in complete inactivity. Methyl groups in positions 5 or 8 are unable to restore this again. The action of vitamin B_2 is therefore like that of vitamin A, bound to an exactly circumscribed constitution and configuration.

This specificity makes itself felt not only against higher organisms but also against bacteria. Elvehjem and my former collaborator Strong* were recently able to show that of all the flavins tested, only lactoflavin, 6-methyl-9-(1′,*d*-ribityl)-isoalloxazin, 7-methyl-9-(I′,*d*-ribityl)-isoalloxazin, and 6-methyl-7-ethyl-9-(I′,*d*-ribityl)-isoalloxazin can stimulate the growth of lactic acid bacteria, i.e. the same flavins which are effective in experiments on rats. On the other hand, flavins with other sugar residues, and also the arabinose compounds, had no effect at all.

Up to the present only *one* natural vitamin A has been found as a decomposition product of carotene - the axerophtol mentioned above. Nevertheless, various observations (Heilbron, Morton *et al*.) suggest that in certain fish-liver oils, e.g. those of fresh-water fishes, perhaps still other substances occur with a vitamin A action, whose absorption spectra show longer waves than that of axerophtol. There is nothing surprising in this possibility, since we know that a vitamin A effect is peculiar to various synthetic decomposition products of β-carotene *with more than 20 C-atoms*, such as the above-

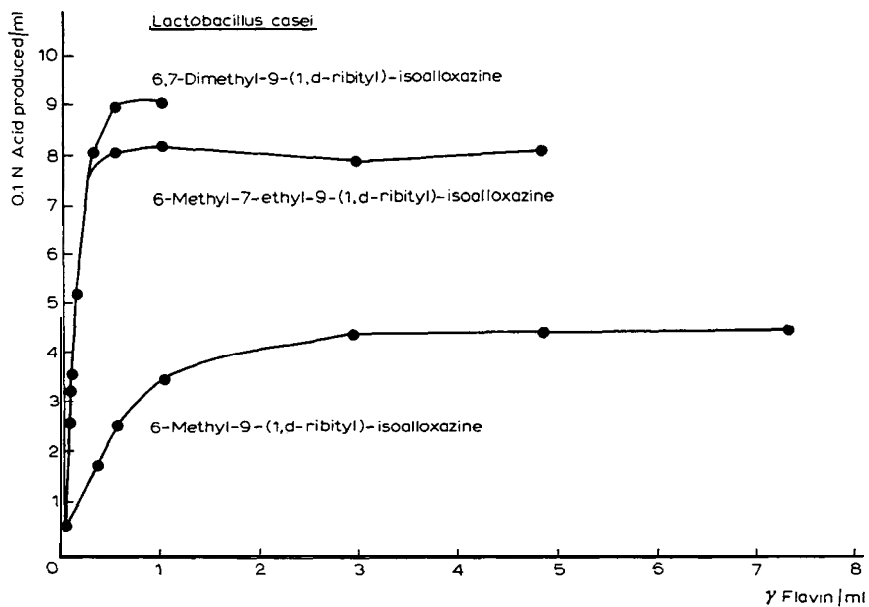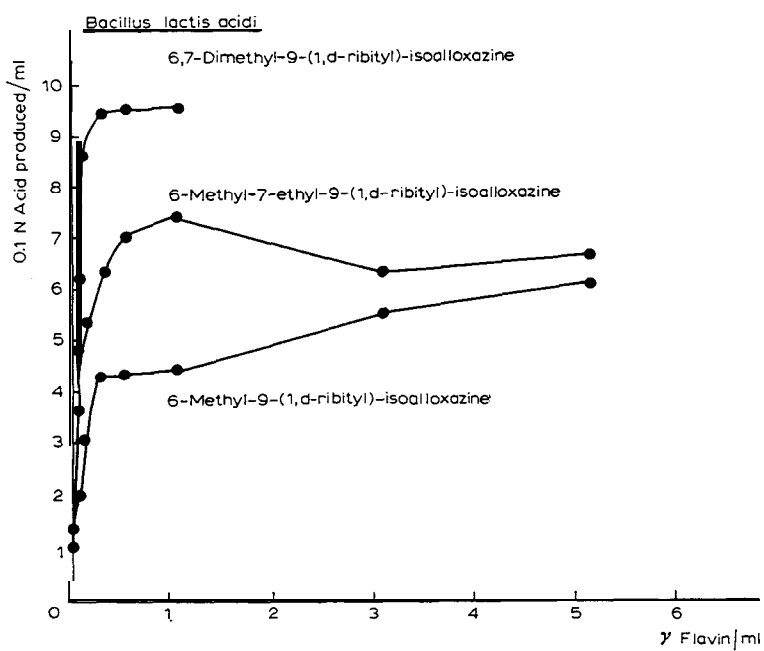* The curves on p. 444 were put at my disposal by Dr. Frank M. Strong.

Fig. I.

Fig. 2.

mentioned apo-carotenals. The thought occurs that Nature too is treading the path which we have been following in the laboratory of late, i.e. in splitting up carotene not only at the central bonds but also at other double bonds, whereby products must result with a longer chromophore system with more double bonds than the ordinary vitamin A possesses. Such substances will be distinguished by absorption spectra of longer wavelengths. Although vitamin A is one of those vitamins whose physiological effects have long been known - Hopkins (1912), Stepp (1911-1912), McCollum, Simmonds, Becker, and Shipley (1922) - even today we are still very much in the dark with regard to the way it intervenes in the cell phenomenon processes. In the cases of vitamin B$_2$ (lactoflavin) and B$_1$ (aneurin), research during the last two years has been more successful in this connection, as the parts played by the first in dehydration reactions and by the second in natural carbohydrate decomposition have been clearly recognized. It is also widely believed today that vitamin A participates in the case of oxidation reduction reactions in the organism. How this intervention happens is, however, still not clearly known.

Vitamin A exercises considerable influence on epithelial formation, and many results of an A-avitaminosis are probably the results of the suspension of the effect of the A-factor which promotes the formation of a healthy epithelium. Thus, for instance, A-avitaminosis resulting from injury to the epithelium is connected with lowered resistance to infections; furthermore, in rats it leads to a chronic oestrus (colpokeratose), i.e. the outer layers of the epithelia of the vagina become horny. Modern surgery is making increasing use of the epithelium-promoting effect of vitamin A, by using cod-liver oil preparations, rich in vitamin A, for helping the healing process in wounds; carotene is believed to have a similar favourable effect.

The action of the A-factor on the eyes is remarkably strong; a vitamin deficiency leads to xerophthalmia, the cause of which is sclerotic change, and to night blindness (hemeralopia), a disease which is often encountered, even in these days, among primitive peoples whose nutrition is deficient. It is most remarkable that the eye is one of the organs most rich in vitamins which we know; not only lactoflavin and vitamin C (ascorbic acid), but also vitamin B$_2$, vitamin K, etc. are present in the eye in considerable quantities. The same applies for the A-factors. Von Euler and Adler have referred to the occurrence of carotenoids in the retina of the eyes of cattle and fish, and G. Wald proved in our laboratory the presence of large quantities of vitamin A (axerophtol) in the retina. According to later in-

vestigations by Wald, vitamin A appears to play an important part in the act of seeing; in the process of exposure to light a pigment, "retinene", combined with a protein and showing a colour reaction with antimony trichloride, is formed from the visual purple. This colour reaction has its absorption maximum at 662-666 mμ. From the retinene, vitamin A is formed (absorption maximum of the antimony trichloride reaction 615 mμ) and finally in the dark the vitamin A is transformed again into visual purple. Eyes kept in the dark contain traces only of vitamin A.

It would seem, therefore, that the chromophore system of the carotenoids is also utilized in the act of seeing, and the animal organism makes varied use of these plants pigments.

Research into the problems connected with vitamin A took place in a roundabout way; as I have explained before, it began with the chemical treatment and elucidation of the composition of the carotenoids. At that time no one imagined that those investigations would become the foundation for vitamin A research. The same circumstances have applied to various other vitamins. One of the most impressive examples, the last to become known, is the story of nicotinic acid amide. This was recognized by Warburg as a constituent of a coenzyme, codehydrase II, and by von Euler as the building material of the cozymase (codehydrase I), in which it occurs together with adenine, pentose, and phosphoric acid:

| Nicotinic acid amide | Adenine | Pentose | Phosphoric acid |
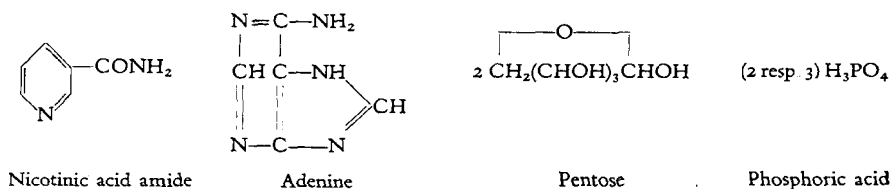
Then, together with O. Warburg, we were jointly able to show that the hydrogen-transferring role, i.e. the central significance, devolves precisely upon this constituent of the two codehydrases in the oxidation-reduction processes.

A hydrogen transference was found to be possible only through such nicotinic acid amide derivatives in which the pyridine nitrogen has a quarternary character. Such compounds (Formula A) are transformed by assimilating the hydrogen split from the substrate into ortho-dihydro derivatives (Formula B), which then pass on the hydrogen to other compounds (S):

In the two codehydrases it is the sugar residue, the pentose residue, which stands in quaternary bond at the pyridine nitrogen of the nicotinic acidamide; the dehydration of the resultant dihydronicotinic acid compound is effected through the chromophore system of the yellow enzyme, i.e. through lacto-flavin (vitamin B_2). The entire dehydration process is represented by the following structural formulae:

As a result of these investigations nicotinic acid amide suddenly became of general interest. It was tried as a remedy in the most varied kinds of diseases, and in doing this Elvehjem and his co-workers discovered that it is the cure for the "black tongue" disease in dogs, on which experiments had been carried out for decades. We are therefore dealing in this instance with a newly discovered supplementary factor in nutrition, a new vitamin.

It has often been said that it is only the first discovery which is difficult and that the ensuing discoveries are usually only the continuations of the first. Perhaps vitamins and carotenoids offer examples of this.

In a lecture which I gave in 1932 I remarked: "The chemistry of the vitamins has made great progress in the last few years; it has overtaken the

chemistry of the hormones and left that of the enzymes far behind. Soon we shall be so far advanced that all kinds of positive statements can be made about the chemical nature of the different vitamins. Then the chemist will be able to hand back the vitamin problem to the physiologist, so that the latter may establish in what way these substances develop their effect in the organism. Once physiology has solved this problem, it will have become enriched by one of its greatest findings."

This prophecy has been fulfilled to a large extent within the short space of five years. The chemical side of the vitamin problem is solved in its essential points; relationships to enzymes were found. It is the task of physiology today to explain the intervention of these agents in the cell processes. As this is, however, a matter of chemical processes, these will in the end probably have to be elucidated by the chemist once again.