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Developments in the Field of Vitamins*

By O. ISLER

Chemical Research Department, F. Hoffmann-La Roche & Co. Ltd., Basel (Switzerland)

The flood of vitamin research in the 1930's followed on the discovery of fat-soluble and water-soluble essential food factors by nutritionists who recognized that their absence caused typical deficiency symptoms in humans and who later devised biological methods for their determination in deficient animals. Between 1930 and 1950 most of the vitamins were isolated, their structure elucidated and then finally synthesized; methods of manufacture were also worked out for most of them. In this research there was no more outstanding figure than PAUL KARRER. As highlights of his work may be mentioned: the isolation of pure vitamin A and the elucidation of its structure, similarly for the carotenes and codehydrases, the structure elucidation of riboflavin and its synthesis, the synthesis of α-tocopherol, the purification of vitamin K, the syntheses of vitamin A acid and of β-carotene and many carotenoids.

The objectives set by PAUL KARRER often led him beyond chemistry into the field of biochemistry, which since 1950 has been definitely dominant in vitamin research. Today the function and reaction mechanisms of the water-soluble vitamins at the molecular level have been to a great extent cleared up. Realizing the potential value of the enormous amount of scientific facts collected over the years, nutritionists and feed specialists have taken a renewed interest in the vitamins.

More recently, the chemist has characterized many naturally occurring active compounds and put them at the disposal of the biologist. He has labelled the vitamins, isolated and synthesized many of the coenzymes, elucidated the stereochemistry of active compounds and the stereoselectivity of enzyme reactions and made a marked contribution to research on biogenesis.

It is intriguing to set down side by side the course of the biogenesis of each vitamin with its technical synthesis and more especially to try to relate their mode of action to their structure.

It still remains for the chemist to determine the nature of the bonds which link the coenzyme with the apoenzyme and, when the necessary methods of structure determination of macromolecules have been evolved, the structure of these protein components themselves.

Table I shows the requirements and deficiency symptoms of human beings, Table II the chronological development of vitamin chemistry.

Table I. Deficiency symptoms and vitamin requirements

Name		Typical deficiency syndrome	Recommended dietary allowances (daily)
A	Retinol	*Night blindness*, xerophthalmia	1.5 mg
D	Calciferol	*Rickets*	0.01 mg
E	Tocopherol	Erythrocyte haemolysis by hydrogen peroxide	30 mg
K	Phylloquinone	*Haemorrhagic diathesis*	1 mg
C	Ascorbic acid	*Scurvy*	75 mg
B_2	Riboflavin	Cheilosis	1.7 mg
	Nicotinamide	*Pellagra*	15 mg
	Folic acid	*Macrocytic anaemia*	0.4 mg
	Biotin	Dermatitis	0.2 mg
	Pantothenic acid	Gastrointestinal disorders	10 mg
B_1	Thiamine	*Beriberi*	1.4 mg
B_6	Pyridoxine	Dermatitis, epileptiform convulsions	2 mg
B_{12}	Cobalamin	*Pernicious anaemia*	5 γ

Table II. Chemical development of vitamins

Name		Purification	Constitution	Synthesis
A	Retinol	1931	1931	1946
D	Calciferol	1932	1936	1959
E	Tocopherol	1936	1938	1938
K	Phylloquinone	1939	1939	1939
C	Ascorbic acid	1928	1933	1933
B_2	Riboflavin	1933	1935	1935
	Nicotinamide	[1935]	1937	1894
	Folic acid	1941	1946	1946
	Biotin	1935	1942	1943
	Pantothenic acid	1938	1940	1940
B_1	Thiamine	1926	1936	1936
B_6	Pyridoxine	1938	1938	1939
B_{12}	Cobalamin	1948	1955	

* Based on a lecture presented at the symposium held in honour of Professor Paul Karrer on his 80th birthday, Zürich, April 18. 1969.

Fat-soluble vitamins

A fat-soluble factor essential for life was first described in 1909. From this factor was developed vitamin A, indispensable for vision and reproduction and different from the fat-soluble vitamins D, E and K with their normalizing effect on skeletal growth, development of tissues and blood coagulation.

Vitamin A

Vitamin A has 2 singular properties: while vitamin A_2 or dehydroretinol is necessary for the vision of the tadpole, the more highly developed form, the frog, requires vitamin A or retinol (Figure 1). Again, there are at least 2 pairs of active forms, the vitamin A acids (retinoic acids) and 11-*cis*-retinals. The vitamin A acids promote healthy growth and normal development of the cell membranes, but have no protective action against blindness and sterility. Indian authors, finding 'epoxyvitamin A acid' on injection to be more active than vitamin A acid, considered it to be nearer the real active form. This was disputed subsequently by American scientists. The sterically inhibited 11-*cis*-retinals are the prosthetic groups of the visual purple of vertebrates, insects and molluscs and, more generally speaking, of all creatures which can see. For studies of absorption, distribution, excretion, and metabolism, vitamin A is most conveniently labelled with ^{14}C in positions 15, 14, 11/12 or 6/7, and particularly easily with tritium in position 11/12.

Substances with vitamin A activity are produced in vivo and in vitro from the provitamin β-carotene in the following way (Figure 2): an oxidase, occurring abundantly in the intestinal mucosa, degrades β-carotene to vitamin A aldehyde, which is converted by reductases to all *trans*-vitamin A to be transported to the active centres. For this purpose, a molecule of vitamin A is thought to be bound in blood to a molecule of prealbumin (molecular weight 50,000) and possibly in addition to a molecule of 'retinol-agglutinating protein' (molecular weight 21,400). For storage, vitamin A is esterified by esterases and retained in the liver preferably as palmitate, which may be changed back to retinol and

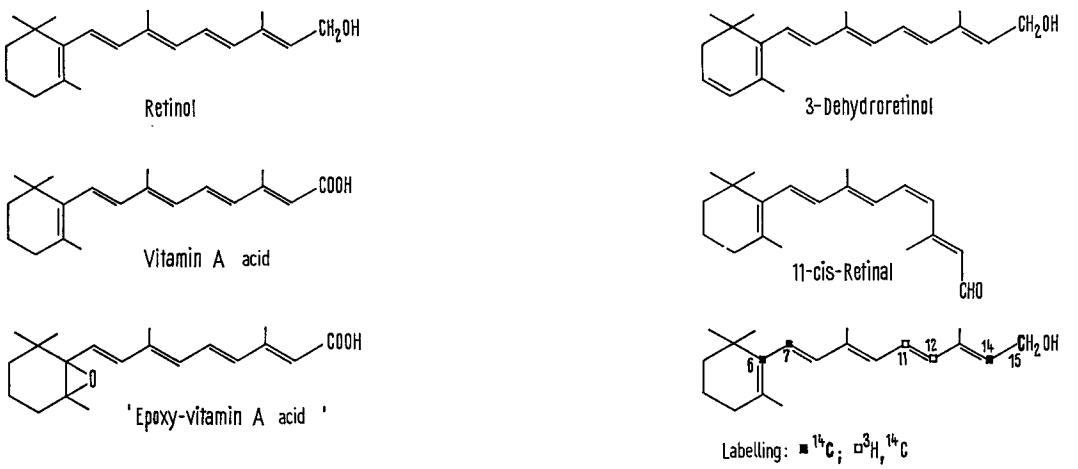

Fig. 1. Formulae of vitamin A compounds and labelling.

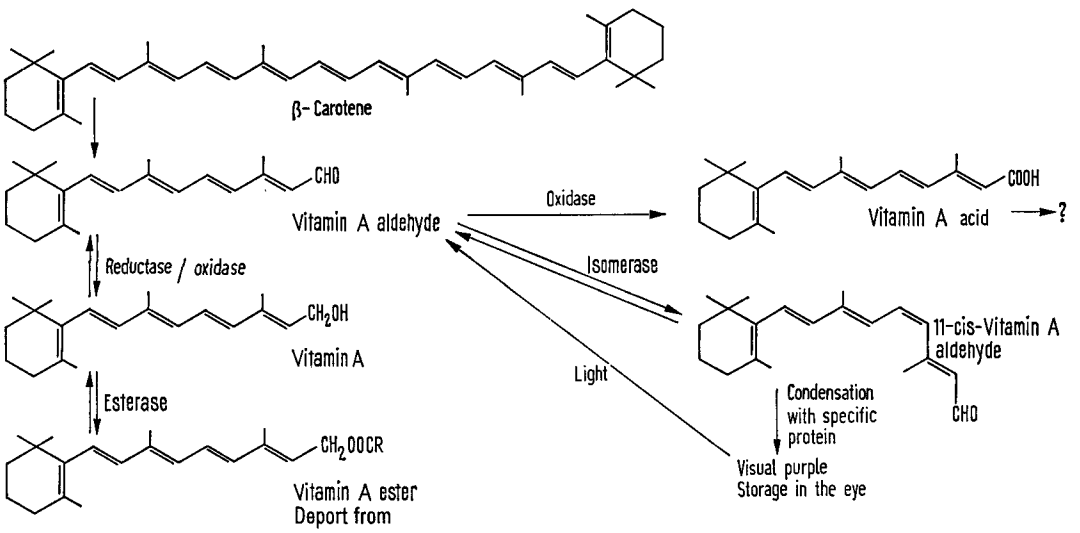

Fig. 2. Vitamin A-active substances from β-carotene.

then to retinal. However, vitamin A acid is formed irreversibly when vitamin A aldehyde is oxidized; surprisingly little is known of the further course of its metabolism. A specific isomerase occurring in the retina catalyses isomerization of vitamin A aldehyde to the 11-*cis* compound, which on condensation with specific protein (opsin) constitutes the visual purple or photoreceptor system of the eye. The American scientist, GEORGE WALD, who in 1932 isolated vitamin A from the ox retina in PAUL KARRER's Institute and in 1967 was awarded the NOBEL prize, demonstrated the transformations on rhodopsin, the visual purple of the retinal rods used for black and white vision, as shown in Figure 3.

By means of light quanta the sterically inhibited 11-*cis* form of the prosthetic group in rhodopsin is transformed into the stable all-*trans* form. This initiates the breakdown of the chromoprotein and the release of the visual impulse. At a lower temperature the breakdown proceeds by way of several reversible intermediate stages of the lumi- and metarhodopsin type. After cleavage, all-*trans*-retinal is isomerized back to 11-*cis*-retinal from which rhodopsin is regenerated by condensation with opsin. According to WALD and MORTON, the vitamin A component in the visual purple occurs as a SCHIFF base, the 11-*cis*-retinylidene residue being bound to the ε-amino group of the lysine part of a decapeptide fragment. Other research workers formulate a protonic SCHIFF base with an aminoethanol linked to lipo-phosphatides in ester-like fashion.

For research into colour vision WALD developed a technique for measuring colour sensitivity of single retinal cones responsible for colour sensation. He found these cones form, with different 11-*cis*-retinylidene chromoproteins, 3 groups of colour receptors possessing sensitivity maxima at 447, 540 and 577 nm. There exists therefore a trichromatic code, thus confirming experimentally the colour theory of YOUNG-HELMHOLTZ.

All industrial syntheses of vitamin A are based on β-ionone (Figure 4).

The Roche total synthesis from acetone proceeds via methylheptenone, dehydrolinalool, and pseudoionone, and twice makes use of a condensation with

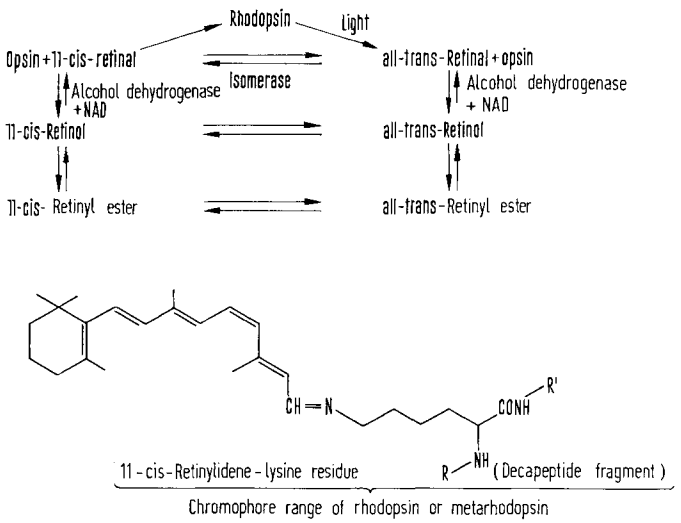

Fig. 3. Vitamin A and visual process according to WALD.

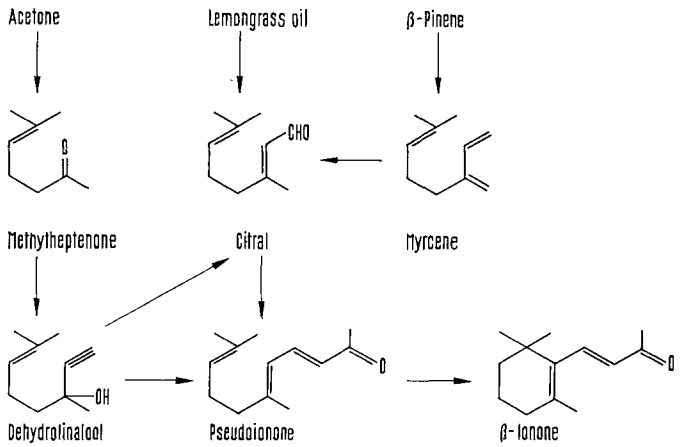

Fig. 4. Technical syntheses of β-ionone.

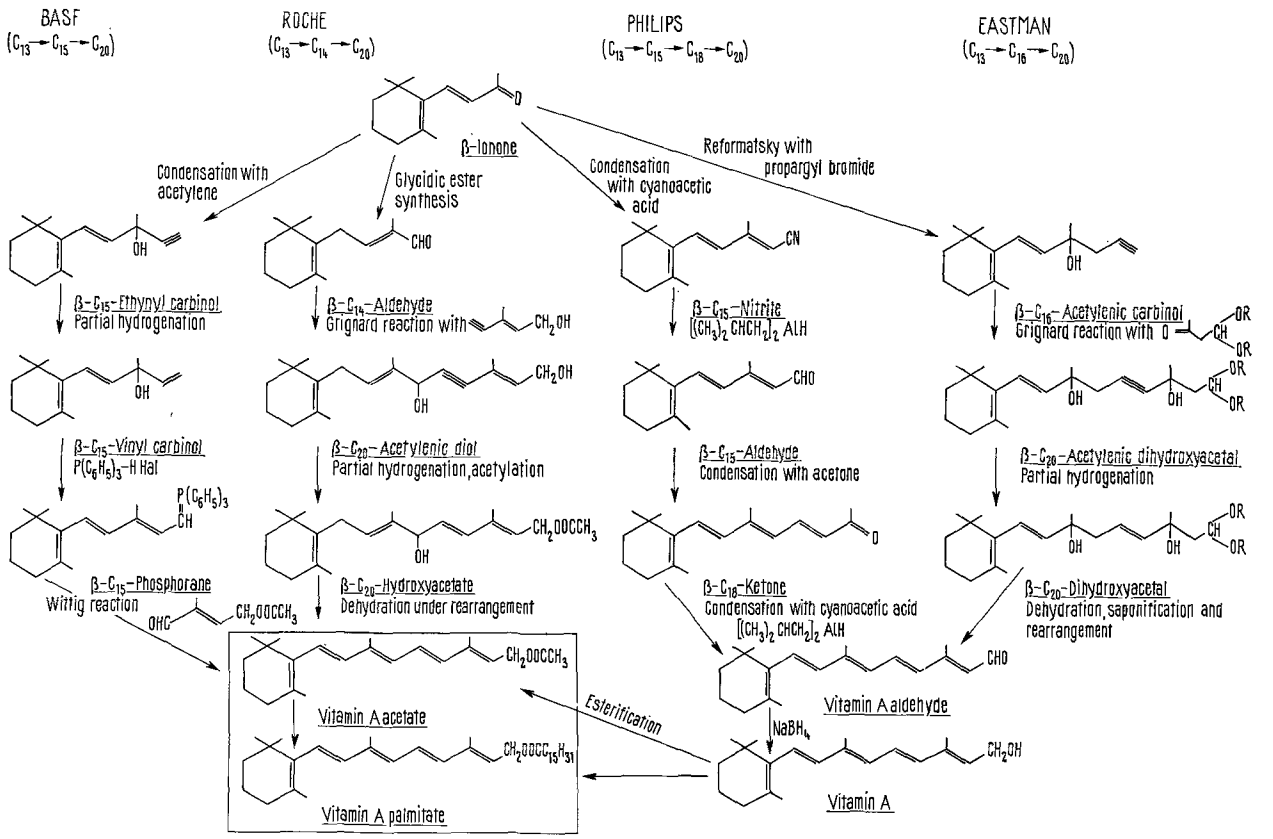

Fig. 5. Technical syntheses of vitamin A esters.

isopropenyl ether discovered and developed by SAUCY and MARBET. Other methods of preparing methylheptenone were developed some years ago by Rhône-Poulenc (from isoprene) and BASF (from isobutylene). Lemongrass oil is no longer an economical source for citral; β-pinene, however, is a good starting material giving via myrcene, citral, and pseudoionone β-ionone.

The technical vitamin A syntheses by BASF, Roche, Philips, and Eastman-Kodak are shown in Figure 5.

From this diagram it can be clearly seen that the procedures of BASF and Roche lead directly to vitamin A acetate, which is fairly stable, crystallizes easily, and is therefore the basis of most commercially available forms. The syntheses by Philips and Eastman first give vitamin A aldehyde, which is reduced to the alcohol and only then esterified to vitamin A acetate or palmitate. In the BASF process, β-ionone is converted to β-C_{15}-vinyl carbinol by acetylene addition and partial hydrogenation; this gives in a WITTIG reaction with γ-acetoxytiglic aldehyde vitamin A acetate. The present Roche procedure is, in principle, identical with the original vitamin A synthesis. We still regard it as the most economical process and this view finds support in the fact that more than two-thirds of the world production is produced in this way.

Sales of natural vitamin A concentrates declined rapidly after synthetic vitamin A was introduced; it is no longer economical.

Vitamin D

Vitamin D_3 (cholecalciferol) is obtained from cholesterol or 7-dehydrocholesterol, vitamin D_2 (ergocalciferol) from ergosterol. Irradiation at low temperature yields precalciferol which rearranges to vitamin D on warming (Figure 6).

Specific labelling of vitamin D_3 was equally successful with cholesterol labelled with ^{14}C in positions 3 and 4, and with tritium in positions 9 and 19 (Figure 7). DELUCA recently isolated 25-hydroxy compounds as active metabolites of vitamin D.

Biogenetically, cholesterol is derived from acetate and mevalonic acid, as shown by its biosynthesis using labelled mevalonic acid, first achieved in the Roche laboratories in 1957 (Figure 8). Dehydrocholesterol is produced by the body from cholesterol, and is mainly deposited in the skin. Exposure of the skin to sunlight or UV-irradiation causes vitamin D_3 to be formed. Therefore the latter can be considered as a genuine vitamin only when the body is inadequately exposed to sunlight, and is otherwise to be regarded as a normal metabolic product of cholesterol.

Vitamin E

Trimethylhydroquinone and phytol or isophytol, which were used in the first syntheses, have to this day remained the basis for the technical production of α-tocopherol (Figure 9). At the time of its first synthesis,

the stereochemistry of phytol was still unknown. Nowadays it is known that the phytol double bond has the *trans*-configuration and the asymmetric centres at positions 7 and 11 have R configuration.

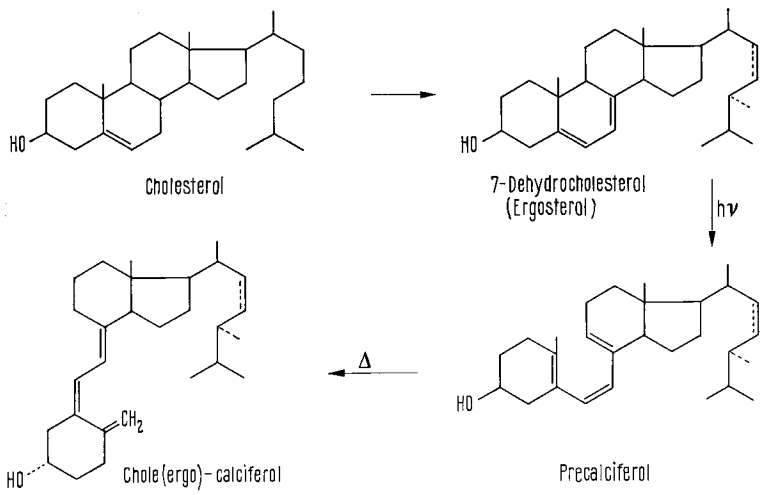

Fig. 6. Technical procedure of vitamin D.

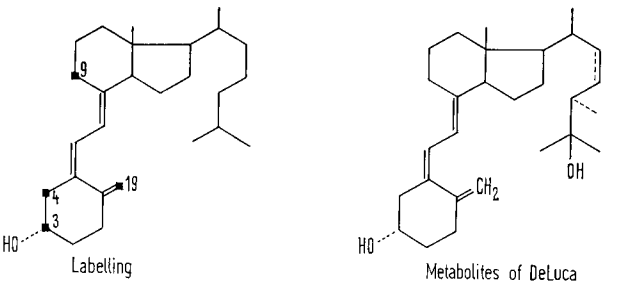

Fig. 7. Labelling and metabolites of vitamin D.

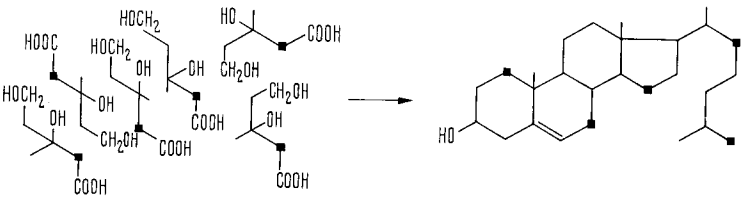

Fig. 8. Biosynthesis of cholesterol from 2-^{14}C-mevalonic acid.

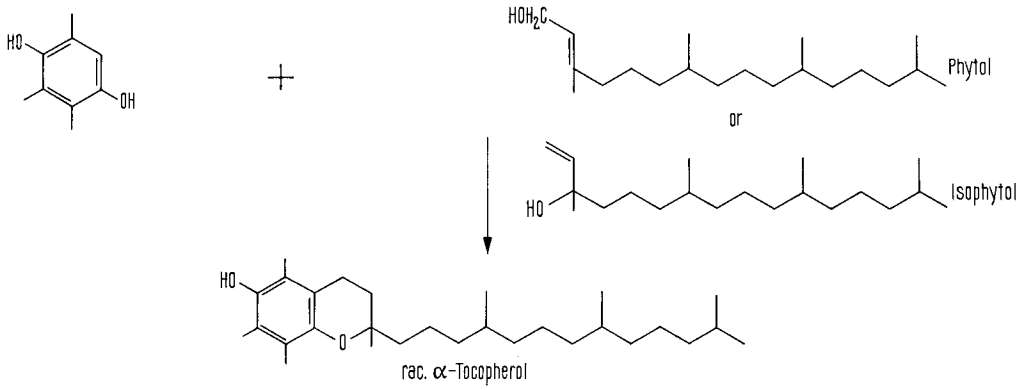

Fig. 9. Technical synthesis of vitamin E.

α-, β- and γ-tocopherol have all R, R, R configuration (Figure 10). The stereochemistry of α-tocotrienol has also been elucidated; the asymmetric centre in position 2 has R configuration while the double bonds of the side chain have *trans*-configuration. Based on biogenetic considerations, the 8 naturally occurring vitamin E compounds could be classed either as α-, β-, γ- and δ-tocopherols or as the corresponding tocotrienols. γ-Tocotrienol is closely related to the plastoquinones and might also be called plastochromanol-3. δ-Tocotrienol is very probably the biogenetic precursor of all the other vitamin E compounds. Its aromatic ring together with the methyl group derive from shikimic acid while the side chain and the C atoms of the hetero ring come from mevalonic acid. The methyl groups in positions 5 and 7 of the α-, β- and γ-compounds are introduced only later with adenosylmethionine.

α-Tocopherol has been labelled in the methyl groups attached to the aromatic ring, in the CH_2 groups of the hetero ring, and in positions 1′ and 2′ of the side chain (Figure 11). On feeding large doses of vitamin E, quinones with partially degradated side chain – so-called SIMON metabolites – are excreted in the urine.

The known transformations in the α-tocopherol series are shown in Figure 12.

The two isomers phytyltrimethylbenzoquinone and 3,4-dehydro-α-tocopherol are mutually transformable. The two isomers obtained by hydrogenation, phytyltrimethylhydroquinone and α-tocopherol, can be oxidized back to the corresponding quinone or chromenol. Tocopherolquinone as well as the tocopherol esters substituted in the methyl group in position 5 can be obtained from tocopherol or from phytyltrimethylbenzoquinone. During the oxidation of d-α-tocopherol to α-tocopherolquinone the configuration of the asymmetric centre C-2 is maintained, whereas in recyclization retention or inversion may occur, depending on the conditions chosen for the reaction. At one time the essentially active substances were thought to be reactive phosphates of the type shown at the bottom right of the scheme, but this idea has now been abandoned. Only the tocopherols and tocochromenols have an effect on the vitamin E-deficient rat. In our opinion,

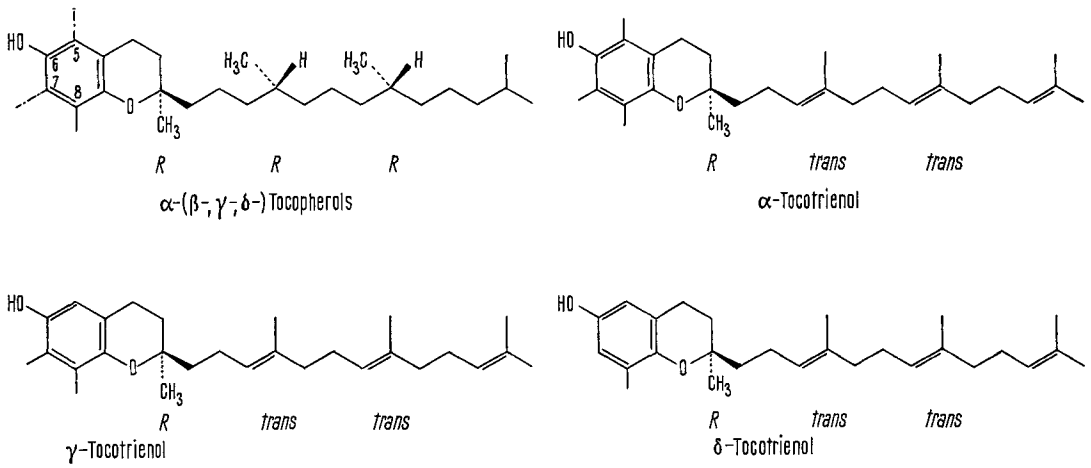

Fig. 10. Absolute configuration and precursors.

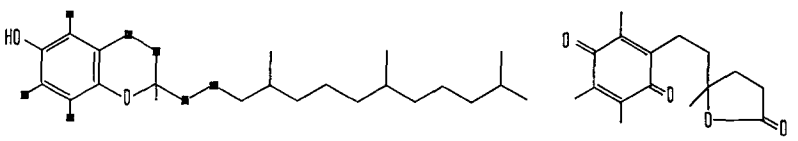

Fig. 11. Labelling of vitamin E and SIMON metabolite.

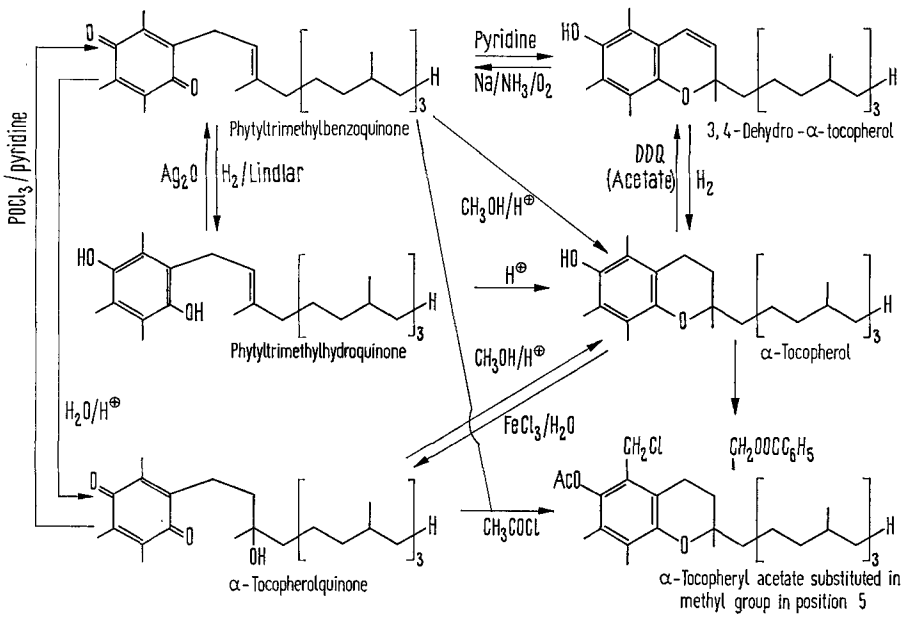

Fig. 12. Transformations in the α-tocopherol series.

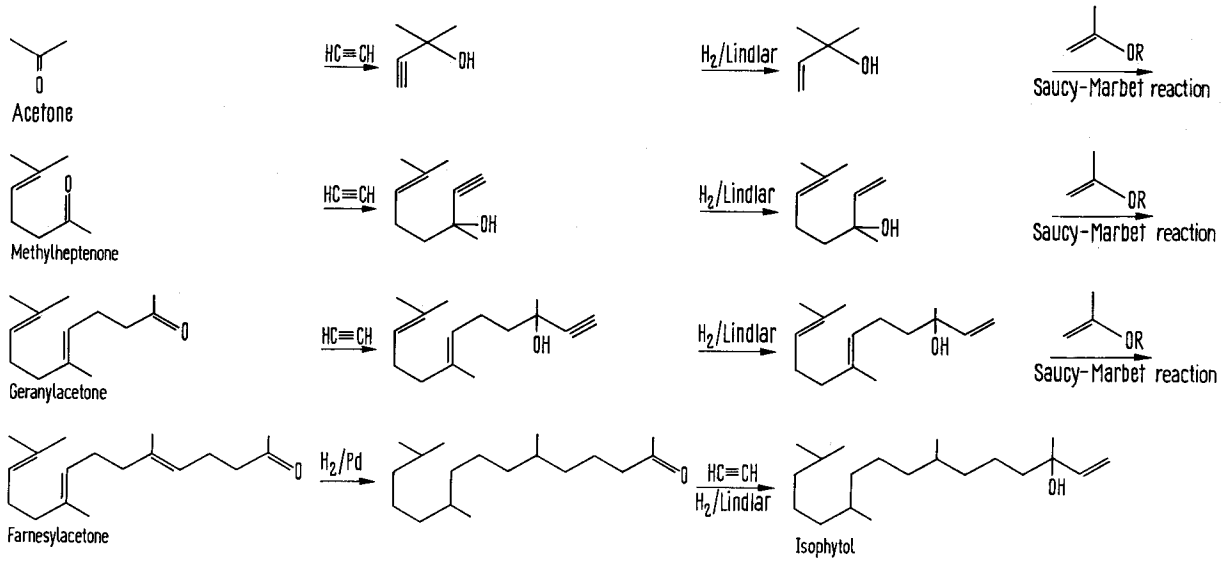

Fig. 13. Isophytol from acetone.

their reversible reducibility has more than just formal significance. Such transformations may well help the biochemist in his elucidation of the enzymatic reactions associated with these compounds.

Nowadays, isophytol is obtained from acetone as shown in Figure 13. The terpene chain is lengthened by 2 + 3 (= 5) C atoms by means of acetylene addition followed by partial hydrogenation of the triple bond using LINDLAR catalyst, and reaction of the resulting vinyl carbinol with isopropenyl ether according to SAUCY-MARBET to give methylheptenone, geranylacetone, and farnesylacetone. Farnesylacetone is then reduced to the saturated C_{18}-ketone which is converted to isophytol; this can be rearranged to phytol if desired.

Vitamin K

The technical synthesis of vitamin K_1 or phylloquinone starts from 2-methylnaphthohydroquinone and phytol or isophytol (Figure 14). Phylloquinone and phytol both have the *trans*-R, R configuration.

Some of the transformations in the K series are shown in Figure 15 with 3 open and 3 cyclized compounds. Such transformations correspond to the analogous transformations in the α-tocopherol series. Here also, the search for active phosphates attached to C atoms and thought for a time to be intermediate products of oxidative phosphorylation, was unsuccessful.

Labelling is best carried out in the methyl group in position 2, which originates biogenetically from methionine, and in positions 1′,2′ of the side chain (Figure 16). Of the vitamin K_2 or menaquinone compounds, which have also an effect on blood coagulation, the whole isoprenologous series, as well as dihydromenaquinones with partly reduced side chains, furthermore inactive demethyl compounds, probable biogenetic precursors, are known. Little however is known about the biogenesis of the naphthoquinone ring which probably derives from shikimic acid. A urinary metabolite of the SIMON metabolite type is formed from

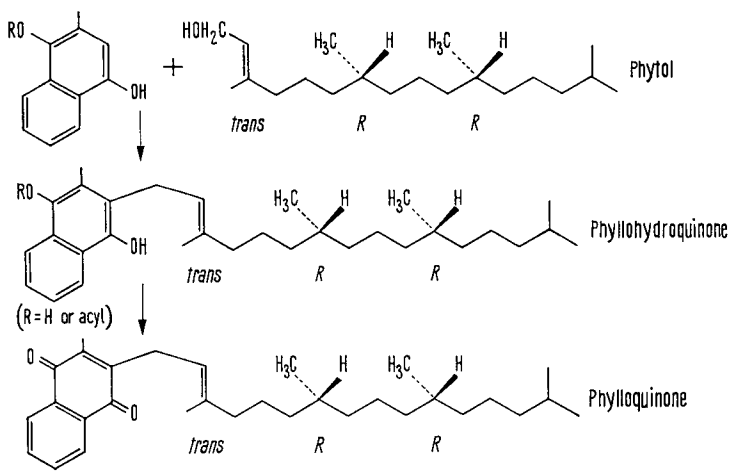

Fig. 14. Technical synthesis of vitamin K_1.

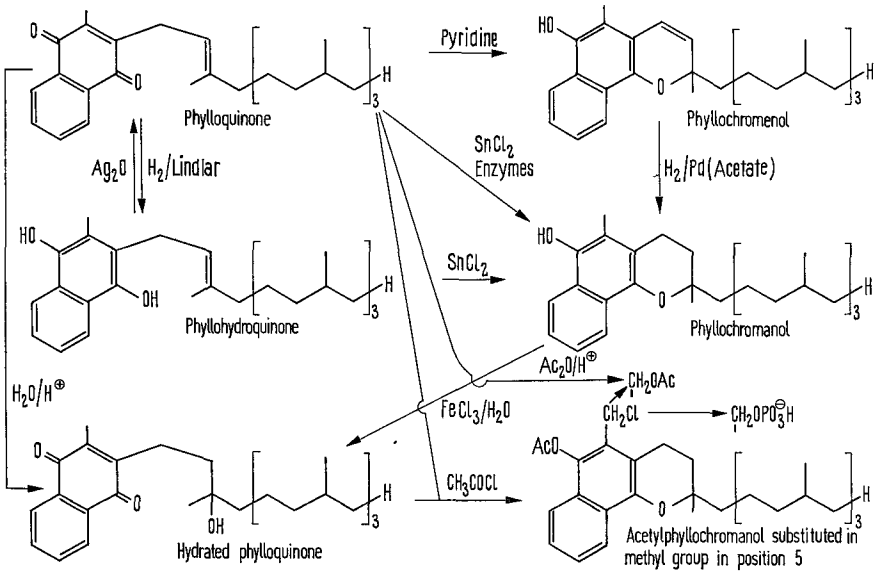

Fig. 15. Transformations in the K series.

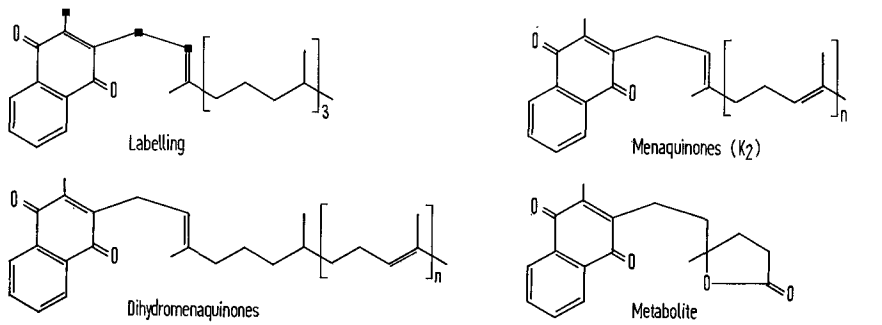

Fig. 16. Labelling, vitamin K_2 compounds and metabolite.

Fig. 17. Related quinones.

phylloquinone and the menaquinones by partial degradation of the side chain.

The ubiquinones and plastoquinones are closely related to vitamins E and K (Figure 17). They are synthesized in a similar way, show very much the same transformations and give analogous urinary metabolites. Biogenesis of the terpenoid side chains always starts from mevalonic acid, that of the ring from shikimic acid. In the case of ubiquinone, the methyl of the methoxy groups and that in position 5 originate from methionine, while in the case of plastoquinone one ring-methyl originates from methionine and one from shikimic acid.

Comparison of total synthesis and biosynthesis

A technical synthesis is naturally different from the biosynthesis. This difference is particularly marked in the case of vitamin A.

The total synthesis (Figure 18) from acetone proceeds via methylheptenone and dehydrolinalool and then, on the one hand, via dihydropseudoionone, isophytol and phytol to vitamins E and K, and, on the

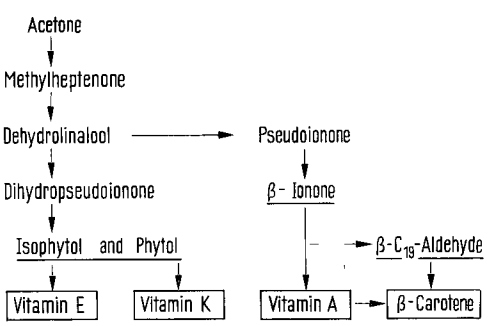

Fig. 18. Total synthesis.

other, via pseudoionone and β-ionone to vitamin A. β-Carotene is synthesized preferably via β-C_{19}-aldehyde, partly also from vitamin A.

Biosynthesis (Figure 19) from acetyl coenzyme A goes via mevalonic acid and isopentenyl pyrophosphate to C_{20}-tetraprenyl pyrophosphate, and from this either to vitamins E and K, or by way of dimerisation to the colourless C_{40}-carotenoids, from which microorganisms and plants form provitamins of the β-carotene type which are then broken down in the animal to vitamin A.

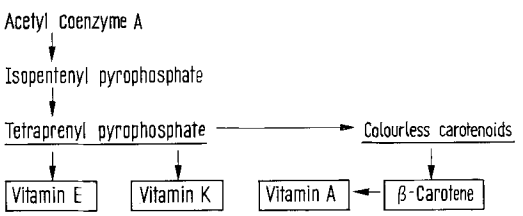

Fig. 19. Biosynthesis.

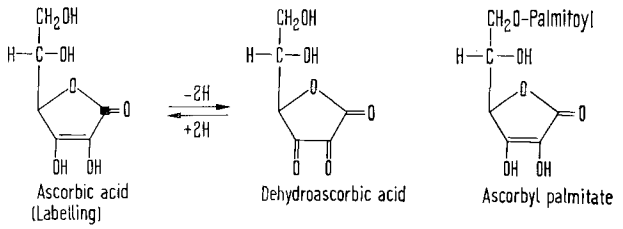

Fig. 20. Vitamin C.

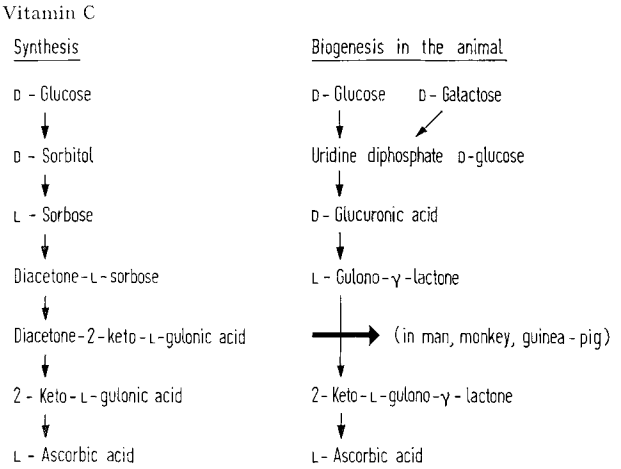

Fig. 21. Technical synthesis and biosynthesis of vitamin C.

The ubiquinones play a part in hydrogen transfer and this may also be true for the tocopherols, phylloquinone and menaquinones.

Water-soluble vitamins

The water-soluble vitamins C, B_2 and nicotinamide are also involved in hydrogen transfer systems.

Vitamin C

Vitamin C or ascorbic acid may be described as the enolized γ-lactone of 2- or 3-keto-L-gulonic acid (Figure 20). It belongs to the biochemical redox systems and can pass reversibly into dehydroascorbic acid. Ascorbyl palmitate is available commercially as a fat-soluble active form.

The technical synthesis (Figure 21) starts from D-glucose and proceeds via sorbitol, sorbose, diacetonide and 2-keto-L-gulonic acid. Biosynthesis in the animal starts from D-glucose or D-galactose and proceeds via uridine diphosphate D-glucose, L-gulono- and 2-keto-L-gulono-γ-lactone. For the majority of mammals an exogenous source of ascorbic acid is not essential. However, man, monkey and guinea-pig lack gulono oxidase, and owing to this metabolic defect, ascorbic acid has become a vitamin for these species.

Nicotinamide

Nicotinamide (the pellagra-preventive factor) is a constituent of nicotinamide-adenine dinucleotide. In Figure 22 we have the oxidized form ($NAD^{\oplus}$), the reduced form of codehydrogenase I (NADH), and the oxidized form of codehydrogenase II ($NADP^{\oplus}$) which carries an additional phosphoric acid group attached to the adenosine half. During hydrogen uptake the pyridine ring is reduced, being left with only two double bonds and having lost its positive charge. Hydrogen enters stereospecifically into position 4. The side which becomes charged with hydrogen during enzymatic dehydrogenation of ethyl alcohol and lactic acid is called the 'A side', while the other, so-called 'B side', is involved in the addition of hydrogen by glucose dehydrogenase or triose phosphate dehydrogenase.

Biosynthesis of nicotinamide starts from tryptophan (Figure 23). In the presence of a vitamin B_6 deficiency, the transition from 3-hydroxykynurenine to 3-hydroxyanthranilic acid is markedly reduced.

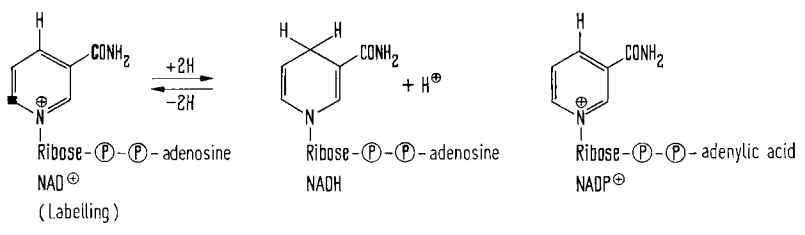

Fig. 22. Codehydrogenases.

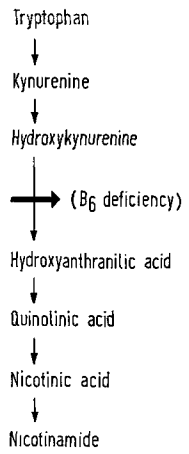

Fig. 23. Biogenesis of nicotinamide.

Vitamin B_2

Vitamin B_2 or riboflavin can be obtained by condensation of an aromatic ribitylamino compound with alloxan (Figure 24). Labelling with ^{14}C is most easily carried out on alloxan prior to this condensation. Biosynthesis starts from ribitylglycine, the pyrimidine ring being next added, followed by the xylyl ring, this latter by means of a double condensation with diacetyl.

Riboflavin is part of the prosthetic groups of approximately 60 known flavoproteins. Some of these, like WARBURG's 'yellow enzyme' are formed from flavin mononucleotide (FMN), the isoalloxazine system acting as a reversible redox system (Figure 25). The probable position of the protein component is shown in the re-

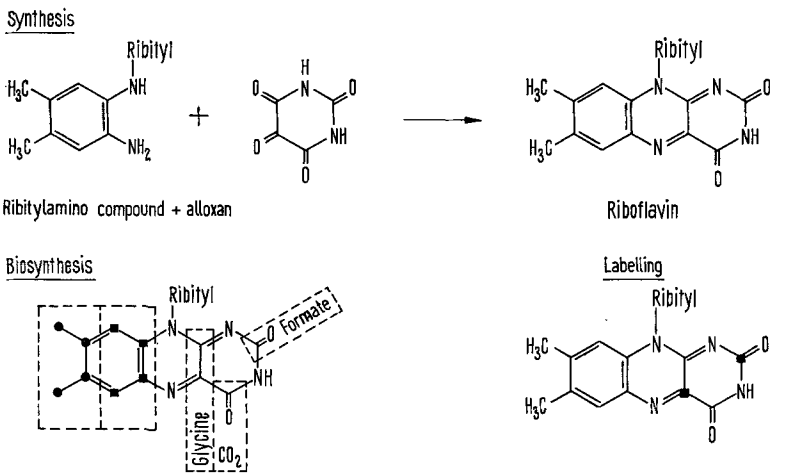

Fig. 24. Synthesis, biosynthesis and labelling of vitamin B_2.

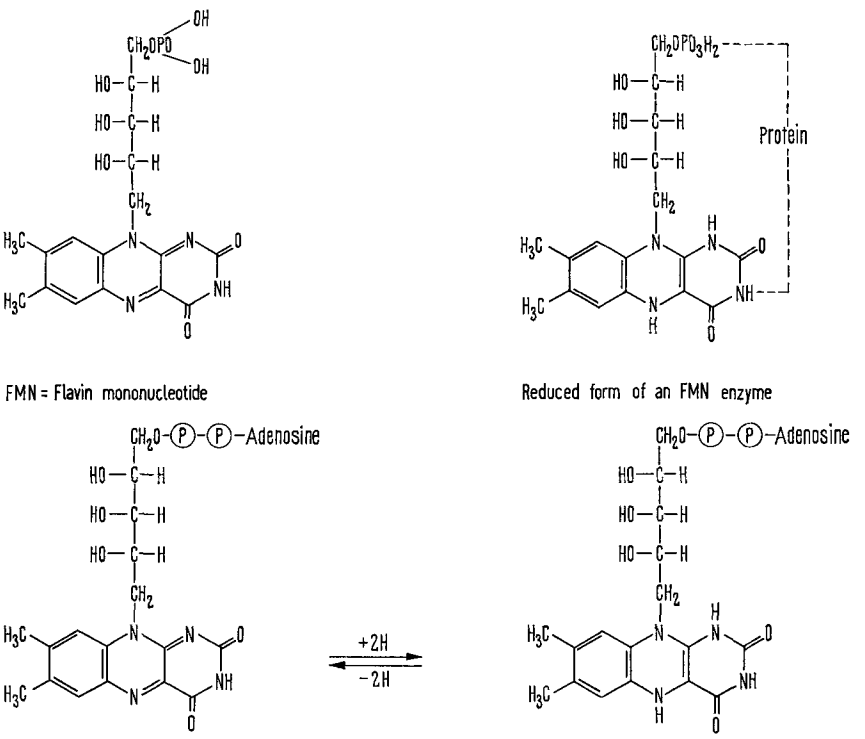

Fig. 25. Riboflavin coenzyme and enzyme.

duced form. Most flavoproteins contain flavin-adenine dinucleotide (FAD) as a prosthetic group. Several flavoproteins take part in the respiratory chain, one of them transferring hydrogen from nicotinamide-adenine dinucleotide (NAD) to ubiquinone. Many flavoproteins contain 'non-haem iron'. There are also flavoproteins capable of transmitting electrons. Research into the flavoproteins is well under way.

Relatively high concentrations of vitamin B_2 are present in the retina, cornea and lens but nothing is yet known of the exact function of vitamin B_2 in the eye.

Folic acid

We now pass from the coenzymes of oxidoreductases to the coenzymes of C_1 metabolism.

Folic acid (Figure 26) is formed both chemically and by biosynthesis from pteroic acid and glutamic acid.

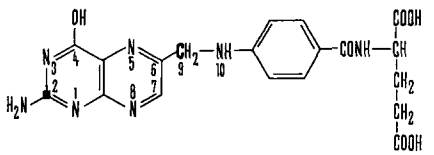

Fig. 26. Folic acid.

Biogenetically, the pterin part should originate from neopterin, the *p*-aminobenzoic acid from shikimic acid, and glutamic acid from histidine. Tetrahydrofolic acid is the biochemically active form (Figure 27).

The 'activated formaldehyde' is probably first bound to N^{10} as a hydroxymethyl group, but easily makes a bridge to N^5, so that N^5, N^{10}-methylenetetrahydrofolic acid is formed. Enzymatic dehydrogenation transforms this into N^{10}-formyltetrahydrofolic acid, the 'active formate'. Serine, which in a reversible reaction can release the β-carbon atom, is an important donor for the hydroxymethyl group. Histidine and glyoxylic acid are examples of donors for 'active formate' which is used e.g. for the synthesis of purines.

The two folic acid antagonists aminopterin and amethopterin inhibit cell division and have a cytostatic effect (Figure 28).

Following on the recent preparative synthesis of biopterin and neopterin in the British Roche laboratories (Figure 29), their influence on the enzymatic hydroxylation of aromatic amino acids is now being examined.

Biotin

Biotin with its condensed ring system of two five-membered rings is obtained from a dibenzyldi[illegible] carboxylic acid anhydride or the corresponding thio-

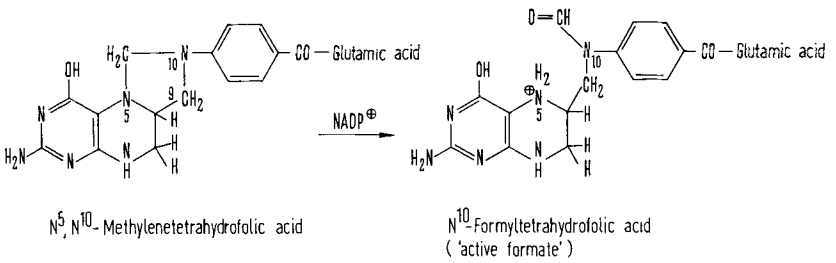

Fig. 27. Active substances for C_1 transfer.

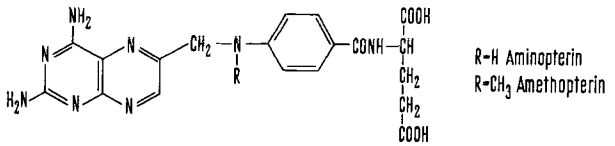

Fig. 28. Folic acid antagonists.

lactone by addition of the side chain (Figure 30). The absolute configuration of biotin was recently established by determining its X-ray structure. Its biosynthesis is probably based on cysteine, pimelic acid, and carbamyl phosphate. Labelling with ^{14}C is most suitably ob-

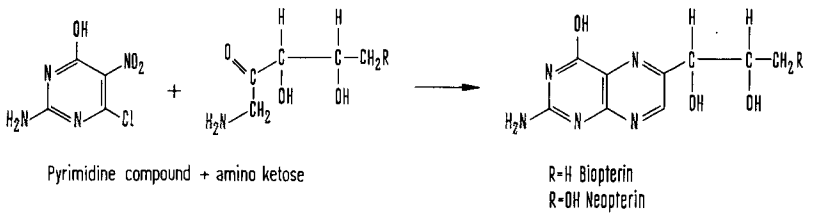

Fig. 29. Synthesis of neopterin and biopterin.

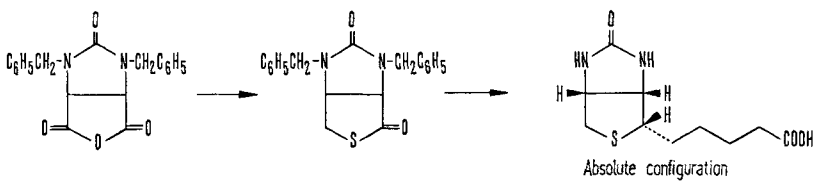

Fig. 30. Synthesis and absolute configuration of biotin.

tained in the end carboxyl group or in the CO group of the ring system.

Biotin is bound to its apoenzyme by the ε-amino group of a lysine residue. With the help of ATP, the biotin enzymes react with carbon dioxide to give the active form of carbon dioxide which is used for α-carboxylations, conjugated α-carboxylations, and transacetylations in purine, fatty acid, and urea syntheses (Figure 31). The formation of malonyl CoA from

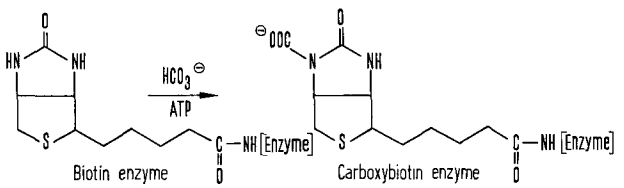

Fig. 31. CO_2 transfer.

acetyl CoA is a particularly important instance in fatty acid metabolism, which has been elucidated by FEODOR LYNEN who advised us in establishing Table III.

Pantothenic acid

Pantothenic acid is obtained by condensation of β,β-dimethyl-α,γ-dihydroxybutyric acid lactone with β-alanine and is most suitably labelled in the carboxyl groups with ^{14}C (Figure 32). With cysteamine it forms

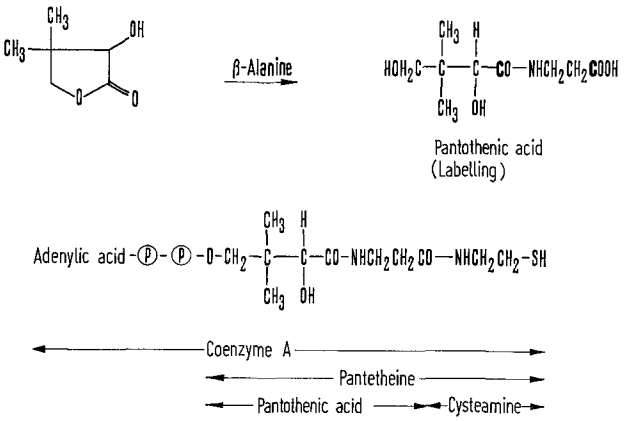

Fig. 32. Pantothenic acid and coenzyme A.

pantetheine, and the latter together with adenylic acid pyrophosphate forms coenzyme A, which is responsible for all enzymatic transfer of acyl groups (CoA = coenzyme of acylation). In this process, the SH group of cysteamine is the active group. Acetyl CoA, the 'activated acetate', is the most important CoA compound. In addition to its function of fatty acid transfer, there should also be mentioned the transfer of succinyl, glutaryl and benzoyl groups. Chemically, these are very reactive thioesters with a high group transmission potential. Coenzyme A is indispensable for the biosynthesis of fatty acids, terpenes, steroids, as well as for fatty acid oxidation.

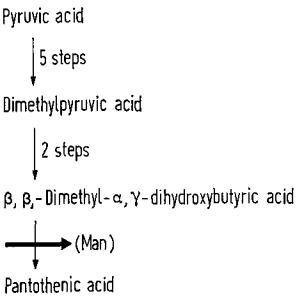

Fig. 33. Biosynthesis of pantothenic acid.

Biosynthesis of pantothenic acid starts from pyruvic acid, and in man is blocked at the last step (Figure 33).

Vitamin B_1

The synthesis and biosynthesis of vitamin B_1 or thiamine are somewhat similar (Figure 34). One technical procedure starts from the so-called GREWE diamine, carbon disulfide and chlorohydroxypentanone. Labelling is most usefully done with CS_2. In its biosynthesis a pyrimidine pyrophosphate is coupled with a thiazole phosphate to give thiamine phosphate.

The pyrophosphate is the active form (Figure 35). The active aldehyde groups which are formed by thiamine pyrophosphate enzymes from β-ketocarboxylic acids, are bound to C-2 of the thiazole ring. 'Active acetaldehyde' is the most important of them all, and is

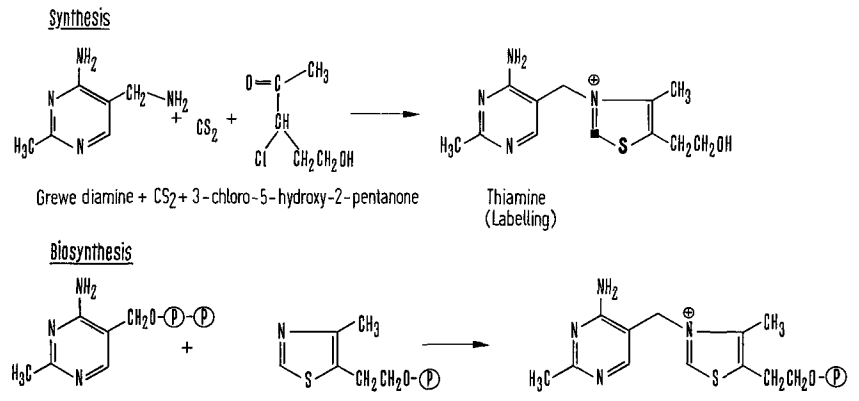

Fig. 34. Synthesis and biosynthesis of vitamin B_1.

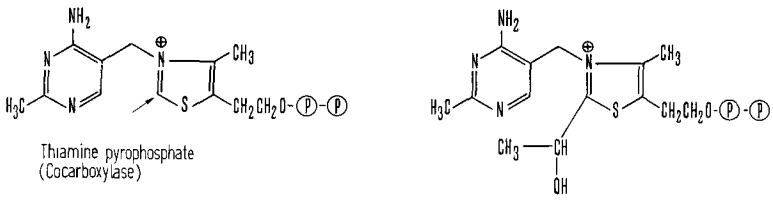

Fig. 35. Cocarboxylase and 'active acetaldehyde'.

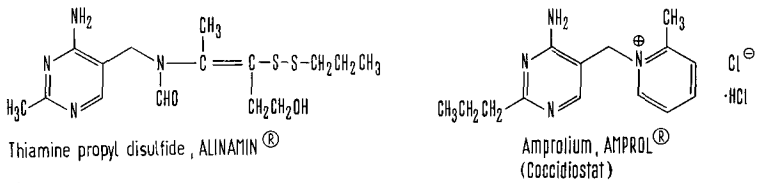

Fig. 36. Compounds related to thiamine.

formed from pyruvic acid. An important function is the transfer of the active aldehyde by the help of lipoic acid, which acts as the oxidant, in the form of formyl, acetyl, succinyl, or generally acyl, to coenzyme A. In the course of the transketolase reaction where thiamine pyrophosphate participates in the pentose metabolism, it acts as a glycolaldehydetransmitting coenzyme.

Some derivatives of thiamine, e.g. thiamine propyl disulfide, are among the most widely used medicines in Japan (Figure 36). Amprolium has been developed as a vitamin B_1 antagonist, and is added to chicken feed as a coccidiostat.

Vitamin B_6

Nowadays, vitamin B_6 or pyridoxine is obtained by DIELS-ALDER condensation of cyano- or alkoxyoxazoles with a 'dioxepene' (acetal from 1,4-dihydroxybutene) (Figure 37). ^{14}C labelling is most suitably performed by labelling 1,4-dihydroxybutene.

Although its biosynthesis has not been elucidated, the reversible transformations of pyridoxine, pyridoxal, pyridoxamine and all their 5'-phosphates into one another in the organism are well known (Figure 38). Pyridoxal phosphate occupies a central position in the amino acid metabolism, and at the same time it

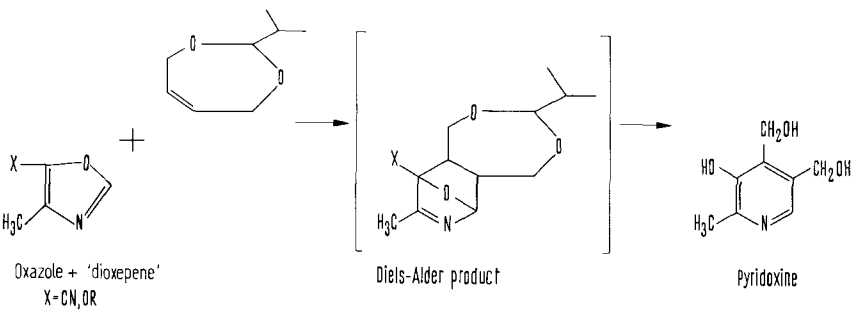

Fig. 37. New syntheses of vitamin B_6.

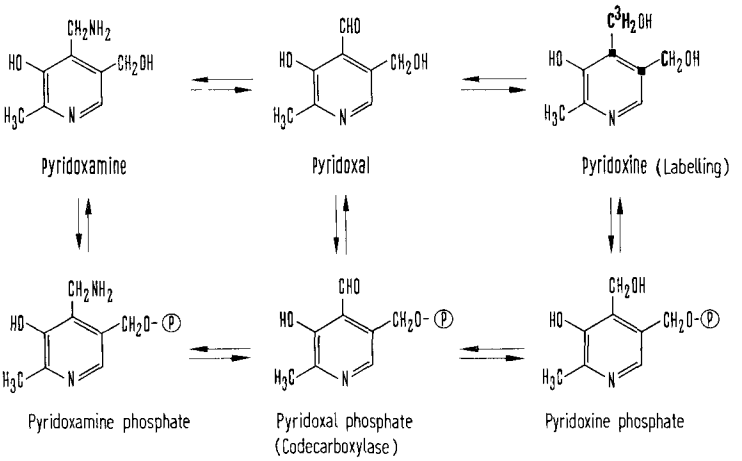

Fig. 38. Transformations in the pyridoxine series.

represents the prosthetic group of amino transferases, decarboxylases, lyases and racemases.

All the different kinds of enzymatic action are based on one intermediate product of the type of a SCHIFF base, the specificity being determined by the different apoenzymes (Figure 39). The numerous functions of the pyridoxal-dependent enzymes show on the one hand how nature makes use of the same prosthetic group for many purposes and, on the other, how important it is to determine the structure of the protein components to arrive at a clearer understanding of the reaction mechanisms involved.

Vitamin B_{12}

The structure of vitamin B_{12} or cobalamin is much more complicated than that of any other vitamin. Its constitution and stereochemistry have been elucidated by determination of its X-ray structure. The B_{12} compounds are derived from the corrin ring and have a trivalent cobalt as a complexly bound central atom. The synthesis of dicyanocobyrinic acid ester, as a joint effort by the schools of WOODWARD and ESCHENMOSER, should be completed soon (Figure 40).

Labelling of cyanocobalamin (Figure 41), a commercially available form, is usually done by introducing radioactive cobalt or the ^{14}C labelled cyanide group. Biogenetically, 6 of the methyl groups of the corrin system originate from methionine, while all 4 of the substituted pyrrole rings originate from δ-aminolevulinic acid. Besides cyanide there occurs a further ligand in cyanocobalamin, namely 5,6-dimethylbenzimidazole which is joined to a side chain of the corrin ring in nucleotide-like manner. In hydroxocobalamin, another commercially available form, the cyanide ion is substituted by a hydroxy group, and in B_{12} coenzymes by deoxyadenosine.

The following functions of B_{12} enzymes have been established:

(1) Isomerization of dicarboxylic acids, e.g. a carboxyl shift in the conversion of methylmalonic acid to succinic acid by means of methylmalonyl CoA mutase.

(2) Transformation of 1,2-propanediol into propionaldehyde by diol dehydrogenase.

(3) Opening of S-S bonds by reductase.

(4) Participation in C_1 metabolism via folic acid enzymes.

Function and use of the vitamins

In Table III the biochemical properties of the vitamins are put together.

Retinol produces the visual impulse by isomerization of the 11-*cis*-retinylidene compounds of the visual purple. The different action of vitamin A acid and the reaction mechanism of vitamin D have not been elucidated. There is strong evidence to suggest that hydrogen transfer might be the function of vitamins E, K, C and nicotinamide, while riboflavin causes hydrogen and electron transfer.

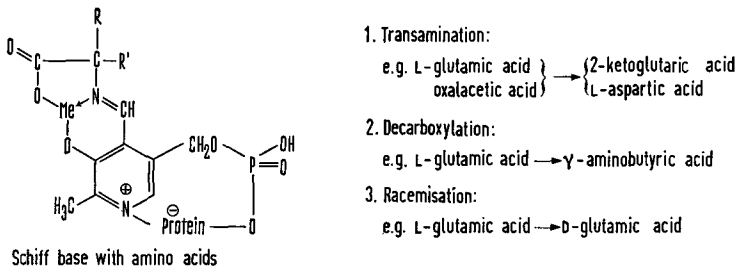

Fig. 39. Function of pyridoxal-dependent enzymes.

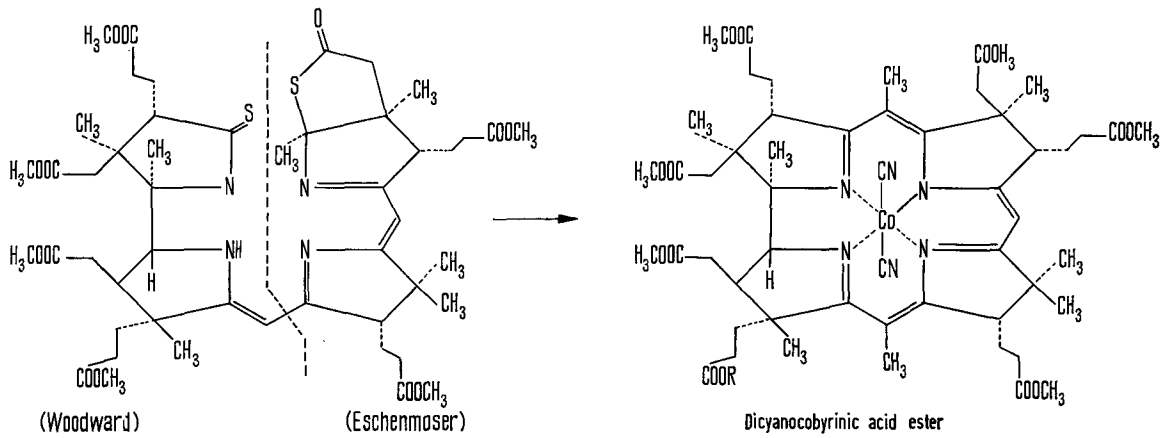

Fig. 40. Plan of synthesis of dicyanocobyrinic acid ester.

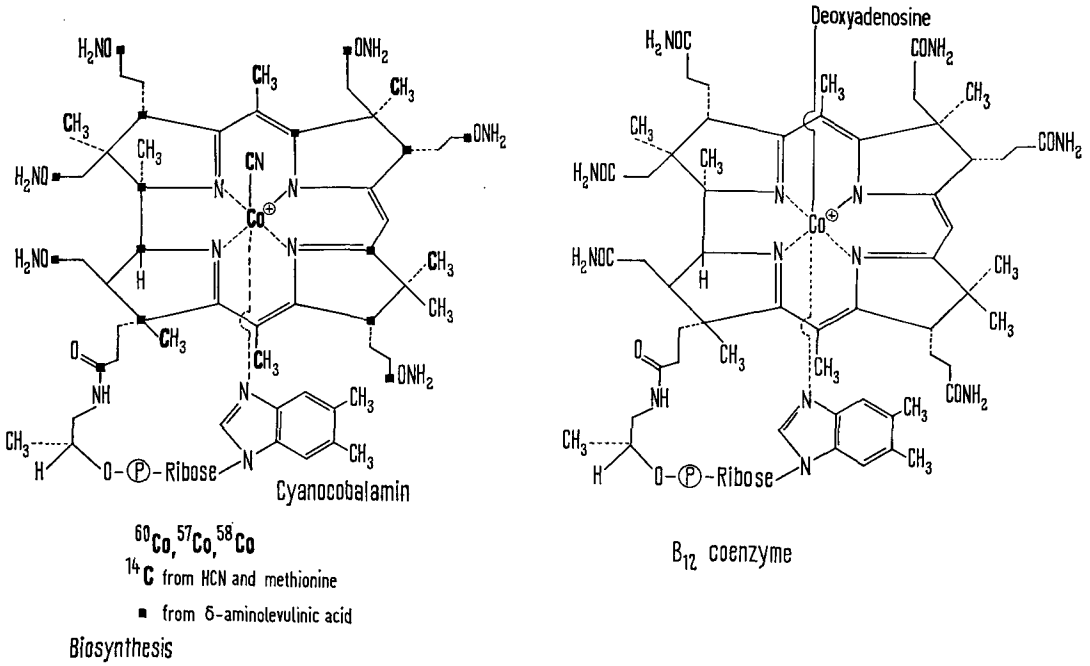

Fig. 41. B_{12} biosynthesis and coenzyme.

Table III. Vitamins and functions of coenzymes

Vitamin	Active form (Enzyme)	Function
Retinol	11-*cis*-Retinal (Visual purple)	**Visual impulse**
Riboflavin	FMN, FAD (Flavoproteins)	**Hydrogen and electron transfer**
Nicotinamide	$NAD^{\oplus}$, $NADP^{\oplus}$ (Transhydrogenases)	**Hydrogen transfer**
Folic acid	Tetrahydrofolic acid (Tetrahydrofolic acid enzymes)	**Formyl transfer ('C_1' metabolism)**
Biotin	Carboxybiotin (Biotin enzymes)	**CO_2 transfer**
Pantothenic acid	Coenzyme A (Transacylation enzymes)	**Acyl transfer**
Thiamine	Thiamine pyrophosphate (Keto acid decarboxylases)	**Aldehyde transfer**
Pyridoxine	Pyridoxal phosphate (Amino acid decarboxylases, deaminases, transaminases, racemases)	**NH_2 transfer (and other functions in amino acid metabolism)**
Cobalamin	B_{12} coenzymes (B_{12} enzymes)	**Isomerization, dehydrogenation, methylation**

As shown, vitamins are the basic constituents of coenzymes which are universally used for most important reactions taking place in all living creatures. Considering that the vitamins are required for the syntheses of bases and pentoses of nucleic acids, for the transformation of amino acids and proteins, for the syntheses of fatty acids and sterols, the question arises whether their selection as ubiquitous active substances was at a period before nucleic acids and proteins determined organized life as we know it today.

The main use of vitamins today is in the food and animal feed industries. Vitaminization of animal feed is now generally accepted; the high quality mixed feeds contain an optimum vitamin supply for the animals, thus preventing the occurrence of deficiency diseases and increasing their resistance to infections, parasites, and unfavourable climatic or living conditions. The following 2 examples from poultry raising demonstrate how vitamin enrichment increases productivity.

Using 2 kg of high production feed in poultry fattening, an increase in weight of 1 kg has been obtained in 8 weeks, while previously twice this amount of feed and time were needed to obtain the same result. The average egg production per hen and year used to be 90, whereas today this has been increased to 220 eggs and more.

Vitamin enrichment of food is of particular importance in those developing countries where there are still children whose health is impaired for life because of vitamin deficiencies. Recent extensive investigations have shown that even in our zone certain groups of the population, e.g. old people in hospitals, are inadequately supplied with vitamins.

Zusammenfassung. Ausgehend von PAUL KARRER's grundlegenden chemischen Forschungen auf dem Vitamingebiet wird ein skizzenhafter Überblick gegeben über die Bedeutung der Vitamine, über Synthese, Markierung, Biosynthese sowie über Funktionen und Wirkungsmechanismen. Dabei wird auch auf wichtige

Zukunftsaufgaben hingewiesen, wie zum Beispiel die Aufklärung der Proteinkomponenten von Vitamin-Enzymen, und schliesslich wird die zunehmende Bedeutung der Vitamine auf dem Lebens- und Futtermittelsektor hervorgehoben.

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Dissociation of Magnesian Calcites

Thermal studies of natural and synthetic calcites have been reported extensively and the dissociation temperature for calcite varies between 840 °C and 990 °C in the literature[1–5]. Lattice parameters of calcite modify with the substitutional impurity in solid-solution within calcite structure. Such systematic variations of lattice parameters with composition of calcite are indicated[6–8]. The present study concerns the changes in dissociation patterns with Mg^{++} in the calcite lattice.

Pure calcite (synthetic), pure dolomite (synthetic), magnesian calcite (synthetic Ca_9Mg_1 composition) from Tem-Pres Research Inc. (USA) and natural magnesian calcite (from Bihar, India) are chosen in the present study. Differential thermal analysis was carried in air with Du Pont thermal analyzer with Al_2O_3 as inert material at 20 °C/min heating rate. The Figure indicates the dissociation patterns for these samples. For calcite (Figure, pattern 1), though the endothermic reaction starts around 600 °C, it is interesting to note that the peak occurs at 798 °C which is lower than the literature values. With artificial mixtures of synthetic calcite and 20% dolomite (synthetic), the thermal pattern indicates no shift in dissociation temperature of calcite (Figure, pattern 2). This may be that both these members have their dissociation in the same temperature range. However, with synthetic

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