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Reviews

The nutritional incidence of flavonoids: some physiological and metabolic considerations

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Summary. Examination of the physiological activity of flavonoids in relation to their antiscorbutic properties shows that some of these compounds, the flavan-3-ols, have a particular nutritional impact and consequently should be distinguished from the rest of the flavonoids and polyphenols. Therefore, the use of the term 'Vitamin P' and 'Bioflavonoids' is also discussed.

Key words. Vitamin P; polyphenols; flavonoids; nutritional role; physiological properties.

Introduction

Fifty years ago, Szent Györgyi used the term 'Vitamin P' to define the antiscurvy activity of some flavonoids which act on the permeability of blood vessels. However, whereas the structures and activities of most vitamins have been elucidated in the course of time, 'Vitamin P' became a vague and imprecise notion. As the years went by, the use of this term changed from the original one, defining an antiscurvy factor, and was transformed into a pharmacological entity gathering all substances acting on the permeability of blood vessels. In the fifties, the Joint Committee on Nomenclature of the American Society of Biological Chemists and the American Institute of Nutrition even rejected vitamin P as a nutritional concept. The substances presenting this particular vascular activity, 'vitamin P-like' substances, have a polyphenol structure. Most of them belong to the group of flavonoids (fig. 1) but they show considerable structural, biochemical and pharmacological diversity. However, advances in flavonoid research have made it possible to distinguish among those substances a category of compounds showing structural unity and reacting from both chemical and physiological points of view, in the same way; flavan-3-ols. Can these compounds be considered as nutrients? What is their exact role? In order to answer these questions, we propose to consider the different aspects of the problem including physiological, metabolic and chemical approaches as well as the historical background.

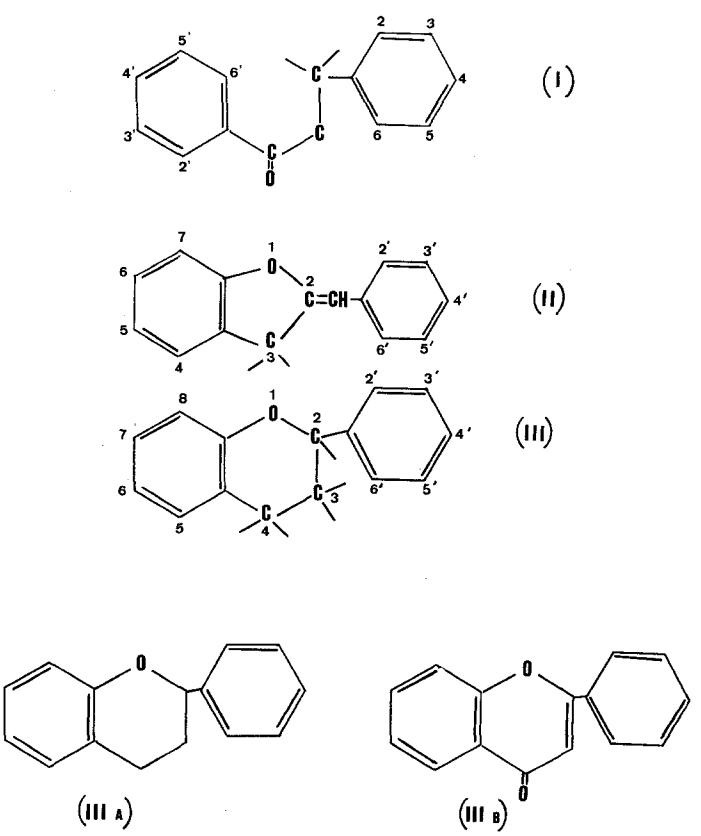


Figure 1. Structures and numerations of the different types of flavonoids. Flavonoids are characterized by a common structure in $C_6-C_3-C_6$: two benzene rings and in between them a C_3 structure which changes with the nature of the flavonoids. The C_3 structure is open in chalcones and dihydrochalcones (**I**) and gives an heterocycle for aurones (**II**) and all other flavonoids (**III**). One can distinguish two structures in the last type of flavonoids (**III**); phenyl-2-chromane (**III** A) and phenyl-2-chromone (**III** B).

Historical context

Originally, the vitamin P concept was inseparable from the notion of a second antiscurvy factor. The antiscorbu-

tic properties of vegetables and fresh fruit have been recognized since the 17th century but it was not until the 1930s that the existence of antiscurvy factors was demonstrated. In fact, it was in 1926 that Zilva extracted from lemon juice a reducing factor which Szent Györgyi identified as ascorbic acid in 1933. At the same time, in 1927, Bezssonoff[15], and Randoin and Lecoq[84] showed that there are two antiscurvy factors in citrus juice which they called 'C_1 and C_2'; these observations were confirmed by Szent Györgyi et al.[2]. Experimental scurvy can be cured by the consumption of citrus juice but not by the mere administration of ascorbic acid. More precisely, the hemorrhagic symptoms of scurvy, due to the fragility of the capillaries, and associated with the enhancement of permeability of the blood microvessels, are cured by paprika or lemon extracts whereas ascorbic acid is ineffective.

The fractionation of natural products showed that the active component is probably a flavonoid[10]. Szent-Györgyi et al. concluded that scurvy is a double avitaminosis; due to the lack firstly of ascorbic acid or vitamin C, then of a vitamin they called vitamin P. (Some consider that the letter P stands for 'permeability' with reference to the vascular activity of the compounds, but it seems more probable that Szent Györgyi chose it to honor his Hungarian origins; P for Paprika, a vegetable widely consumed in Hungary.) Vitamin P was considered to be more particularly involved in the vascular syndrome. However, they observed that vitamin P is only effective in the presence of at least a trace of ascorbic acid[11,12]. So 'Vitamin P' and 'C_2 factor' were, in fact two names for the same nutritional concept. Nevertheless, the difficulty of preparing diets without flavonoids, and of separating the different flavonoids in natural extracts, led to the vitamin nature of the C_2 factor being questioned.

Szent Györgyi et al. attempted to isolate this factor but the 'citrin' they crystallized from the lemon juice was identified as a mixture of hesperidin and eriodictyol glucoside[25]. Afterwards, many investigations[60,79,81,94,95] supported Szent Györgyi's conclusions and confirmed the physiological activities of flavonoids[20,104,106]. Others, on the basis of pharmacological experimentation[57,64] and experiments with deficiency diets[32], deny the existence of vitamin P; however, the validity of these experimental diets is open to discussion. Lavollay, himself, emphasized that the diets were composed of natural substances more-or-less adapted to the animals used (generally guinea pigs) and, under such conditions interpretating the results is generally quite difficult[65]. These criticisms which referred to Parrot and Kursanov's work, might apply just as well to his assistant Fabianeck's semi-synthetic regimes[33], showing that guinea pigs were healthy after thirteen months of these diets was, for this reason, not convincing proof of the inessential nature of flavonoids. Later, other authors[35,36,75] questioned the vitamin nature of polyphenols, basing their arguments on the heterogeneity of the structures acting on blood capillaries, the failure of the attempts to isolate flavonoids in organs and the vascular activity of ascorbic acid[28].

Then the vitamin P nutritional concept gave way to the pharmacological notion of vitamin P-like substances. As Parrot et al.[82] explain, the term 'Vitamin P' is no longer even related to the notion of food deficiency, but has become a pharmacological one; the name of 'P factor' will soon be used for any substance able to increase the capillaries' resistance and to reduce their permeability, without the physiological mechanism of its specific action being examined. The modification of microvessels, which provides experimenters and clinicians with a convenient test, has been artificially isolated from a range of physiological actions. That is why various polyphenols, flavonoids and also coumarins[29,52,57,62] which have no antiscurvy activity but present a vascular activity are classified as vitamin P-like substances, contrary to Szent Györgyi's recommendation: 'It would be a grave mistake to call P a permeability vitamin'.

In order to underline the pharmacological action of flavonoids on vessel walls, various American authors[47,97,98] put forward the term 'Bioflavonoids'. However, in 1968, the US Food and Drug Administration denied that flavonoids were efficient 'for any conditions'[55].

We will have to wait for completely synthetic diets[8,38] to reaffirm the existence of a second antiscurvy factor. Some flavonoids acting with ascorbic acid possess antiscurvy properties. Scurvy is therefore a double avitaminosis. To prevent it, two factors are necessary; on the one hand, a compound, ascorbic acid, that alone neither prevents nor cures scurvy but only delays its appearance or reduces its effects; on the other hand, a cellular factor belonging to the flavonoid group, which does not act by itself on scurvy, in the absence of ascorbic acid[38]. This hypothesis has been developed from experiments carried out on guinea pigs; these animals have lost their capacity to synthetize ascorbic acid and can thus develop scurvy, just as man does. Other studies, carried out on rats, showed that when their diet lacks flavonoids, these animals develop brain edema and subpleural hemorrhages[9]. Other authors[20,28,55,59,74] using physiological and pharmaceutical approaches conclude that flavonoids represent a particular category of compounds which 'without being indispensable for the maintenance of life, furnish an important contribution to the health-promoting properties' and consider them as 'semi-essential food components'[59]. This play on words, as Hughes[55] emphasizes – either a compound is essential or it is not – sheds light on how difficult it is to clarify the exact role of such compounds. The structures and complex reactivity of these substances are involved and, as Kühnau[59] says: 'The complexity of the flavonoid problem in human nutrition already finds its expression in flavonoid chemistry'.

Comparison between physio-vascular activities of anti-scurvy factors and vitamin P-like substances

The many and varied physiological and pharmacological properties of ascorbic acid and vitamin P-like polyphenols cannot all be considered in this text. Therefore only the physiological activities of those compounds which are related to scurvy, as well as the comparison of related metabolic or biochemical mechanisms, will be dealt with.

Scurvy is a deficiency disease whose symptoms vary according to age. The Barlow disease, in babies, is characterized by weariness, anemia and bone lesions (swollen epiphyses especially at the chondrocostal level) with hematomas under the periostea. Adults get cutaneous and intramuscular hemorrhages accompanied by turgescence of the gums which easily bleed. Clinical symptoms of scurvy only appear after several months of a deficiency diet and nowadays rarely occur. On the contrary, hypovitaminoses are more common and their symptoms are not very specific; asthenia, emaciation, headaches, pain in the bones and lower resistance to infections. These various pathological disorders come from the deterioration of mesenchyme tissues, especially due to defects in collagen and dentine, in the formation of which various compounds such as ascorbic acid and some flavonoids are implicated.

Collagen is a fibrillar scleroprotein which is composed of tropocollagen subunits which include high percentages of hydroxyproline and hydroxylysine. These amino acids become glycosylated, and the glycosyl residues contribute to the stability of the molecule. The biosynthesis of the two hydroxy amino acids from lysine and proline residues takes place while the chains are forming and is catalyzed by hydroxylases. The reaction requires the presence of molecular oxygen, ferrous iron and ascorbic acid. The latter has a double role; its presence is required for the efficient functioning of the hydrolase, and it acts as a reducer of Fe(III) to Fe(II)[5, 26]. Thus an ascorbic acid deficiency leads to the formation of underhydroxylated collagens within the fibroblasts. These consequently present abnormal physical properties, and the synthesis of glycosaminoglycans is also disturbed[67]. Such deficiency contributes to the occurrence of hemorrhages during the development of scurvy.

The histological study of blood vessels shows that continuous vessels present a single layer of endothelial cells separated from the adjoining tissue by a basal lamina. Using electron microscopy two areas within the basement membrane can be distinguished; a high-density central area with a fibrillar structure (collagen and tropocollagen) and surrounding it a low density area, in which mucopolysaccharides are present. The formation of these involves ascorbic acid which takes part as 2-sulfate ascorbic acid in sulfate esterification of mucopolysaccharides. An antiscurvy compound deficiency (ascorbic acid or some flavonoids) alters the basal lamina, which becomes irregular; swellings are accompanied by splitting and the basal lamina is easily broken by a mild trauma. Since it acts on the biosynthesis of collagen, ascorbic acid directly influences the integrity of the blood microvessels. Specific enzymes have been used to demonstrate ascorbic acid located within the basal lamina[43].

However, ascorbic acid is not the only substance acting on blood capillaries. Several studies underline the role of polyphenols on the various vascular tissues. Specific tracers have shown that there are polyphenols located in the cytoplasmic membrane of cells of alveolar capillaries; the basal lamina is also, but more lightly, marked[41]. These results support other observations which have shown that (+) catechin is present in the lipid fraction of cytoplasmic membranes[88], and that the flavonoid antiscurvy factor is located within the phosphatidylcholine fraction of guinea pig liver[40]. An early breaking up of the endothelial membranes of capillaries is, moreover, a symptom of a 'C_2 factor' deficiency[39].

Direct effects of flavonoids on collagen have also been noticed. Catechin is implicated in the formation of hydrogen bonds between the collagen chains, in vitro[96]. Also several flavonoids; flavan-3-ols (catechins, monomers and oligomers), flavan-3,4-diols (leucocyanidin), flavonols (quercetin and derivatives), and flavones (fig. 2) have a strong antilathyrogen activity. They inhibit the lysine-oxidase that catalyzes the oxidation of lysine into allysine. Consequently they prevent degeneration due to lathyrogens[27, 47, 48]. The hyaluronidase acting on mucopolysaccharides as well as elastase, a proteolytic enzyme acting on connective tissue, and more generally, the lysosomal hydrolases damaging glycosaminoglycans, are inhibited by flavan-3-ol oligomers[58]. This enzymatic inhibition also concerns prolylhydroxylase[17–19] which is implicated in the hydroxyproline formation, although existing collagens are not modified. These activities appear to be the result of the antioxidizing activity of the flavonoids and their capacity to form complexes with metals, particularly with copper, which is necessary for the efficient activity of the above-mentioned enzymes. A competition for the free-radicals produced by the interaction of Fe(II), ascorbate and oxygen has also been suggested[70]. Therefore an evaluation of the anti-oxidizing activity of flavonoids has recently demonstrated that catechin, in the contrast to flavone, exerts a powerful antilipoperoxidant effect (membrane protective effect) whereas rutin (quercetin-3-rutinoside or quercetin-3-rhamnoglucoside) presents a strong antioxidant effect (radical scavenger)[93].

The action of ascorbic acid and flavonoids on various structural elements of blood capillaries results in physiological effects on blood vessel permeability and vascular tissue resistance (VTR). Both parameters are implicated in very similar blood phenomena and are inversely related to one another. Permeability is evaluated by determination of parietal porosity (involving liquid exchanges and protein diffusion) which can be measured with a dye[78]. VTR tests vascular tissue strength using the mod-

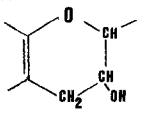
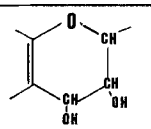
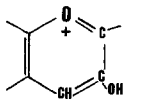
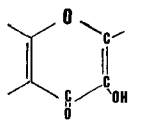
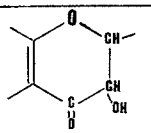
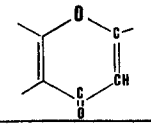
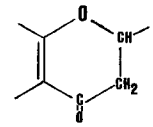
FAMILY	STRUCTURE OF HETEROCYCLE	MAIN SUBSTANCES	
		name	hydroxylation
FLAVAN -3- OLS *catechins*		catechin gallocatechin	5,7,3',4' 5,7,3',4',5'
FLAVAN -3,4- DIOLS *leucoanthocyanidins*		leucocyanidin leucodelphinidin	5,7,3',4' 5,7,3',4',5'
ANTHOCYANIDINS		pelargonidin cyanidin delphinidin	5,7,4' 5,7,3',4' 5,7,3',4',5'
FLAVONOLS		kaempferol quercetin myricetin	5,7,4' 5,7,3',4' 5,7,3',4',5'
DIHYDROFLAVONOLS *flavanonols*		fustin taxifolin	7,3',4' 5,7,3',4'
FLAVONES		apigenin luteolin	5,7,4' 5,7,3',4'
FLAVANONES *dihydroflavones*		naringenin butin eriodictyol	5,7,4' 7,3',4' 5,7,3',4'

Figure 2. The different families of the main type of flavonoids (derived from phenylchromane and phenylchromone structures). The C_3 structure is A–CH_2–CHOH–CHOH–B (flavan-3-ol), A–CHOH–CHOH–CHOH–B (flavan-3,4-diol), A–CH_2–CO–CO–B (anthocyanidin), A–CO–CO–CHOH–B (flavonol), A–CO–CHOH–CHOH–B (flavanolol), A–CO–CH_2–CO–B (flavone), A–CO–CH_2–CHOH–B (flavanone) according Geissman, op. cit. Ribereau-Gayon[87].

ified technique of depression[80] which involves the appearance of small cutaneous hemorrhages (petechiae). The VTR measurement is easier to carry out and more precise than the common determination of blood permeability.

The intraperitoneal administration of antiscurvy or vitamin P-like substances to guinea pigs brings about two types of VTR increase. All the substances increase the average VTR. However, numerous compounds show an early increase after 6 h, which is short-lived (24 h) with an average intensity of 10 to 20 cm mercury elevation, whereas other rarer compounds induce after the first increase a second one characterized by a longer delay (after 48 h), a longer duration (144 h) and a higher intensity (over 30 cm of mercury). Two categories of compounds which act on VTR can be distinguished. The one inducing the VTR monophasic increase, includes besides epinephrine and other catecholamines, numerous polyphenols, quercetin and its derivatives (e.g. rutin), flavones and more generally, flavonols, chalcones, isoflavones and coumarins[44]. Among the substances with a biphasic action on VTR are ascorbic acid, flavan-3-ols, catechins[83], leucocyanidin[73] and natural extracts which contain gallocatechin[85] and have been shown to possess antiscorbutic properties. In contrast, high polymerized catechins (phlobaphens) have the opposite effect, decreasing VTR and favoring scurvy[38].

Hence, there are two ways in which blood capillaries are affected; a general action brought about by vitamin P-like substances, and a more specific activity by antiscurvy factors. The first VTR can be qualified as epinephrine-like. Catecholamine catabolism involves catechol-O-methyl transferase (COMT)[4]. Simple polyphenols (pyrogallol) or flavonoids (myricetin) are non-competitive inhibitors for COMT[21]. A different inhibiting mechanism, involved notably in mammalian metabolism of flavonoids by ring-fission or appearance of 3'-O or 3-O methyl esters, has also been observed; a competitive inhibition between orthodiphenols and catecholamines. Some of the methyl groups of S-adenosyl-1-methionine

are taken up by flavonoids and consequently, catecholamine catabolism is partly inhibited [42]. Ascorbic acid has also been found to act as a substrate for COMT. A methylation on position 2 has been seen in vitro [16, 23] as well as in vivo and induces a secondary metabolic conversion characterized by the appearance of 2-methyl-ascorbate in the urine [45]. Therefore it protects catecholamines (well known for their peripheral vasoconstrictor properties) as polyphenols do.

The VTR second increase, however, seems to be related to a specific activity of compounds that have been proved to be directly involved in effects on the molecular structure of blood capillaries. Another hypothesis [77] suggests that the biphasic elimination of catecholamines (first maximum after 6 h, second one between 12 and 48 h) is probably due to the enterohepatic cycle or to a late release of polyphenols [31]. In such a case, the VTR biphasic increase ought to be considered as the result of interactions between catecholamines, flavonoids and ascorbic acid. These VTR results are confirmed by measurements of blood vessel permeability, which decrease with (−)epicatechin and flavan-3-ol oligomers [58]. Effects of other flavonoids on the vascular system have been pointed out recently and are connected with the above phenomena. However, precisely what effect is observed depends on flavonoid concentration, molecular structure, spacial configuration and electronic characteristics [14].

Several flavonoids affect erythrocytes aggregation and sedimentation [89]. Some inhibit blood platelet aggregation and decrease platelet adhesion to glass. This can take place at different levels. Catechin and naringenin (although they induce a strong platelet fixation on collagen) are weak inhibitors of ADP or thrombin-dependent aggregation [14]. It has also been suggested that the effect on cell aggregation could involve calcium [90]. Effects on lipid metabolism have also been noticed; on cholesterol [6], and on the precursor of prostaglandins, arachidonic acid [7, 61]. In vitro, different flavonoid compounds exhibit a wide rang of effects on arachidonic acid metabolism [103]. (+)Catechin, and in a weaker way (−)epicatechin inhibit prostaglandin synthetase whereas rutin and various other derivatives inhibit lipoxygenase, and others, such as luteolin and dihydroxyflavone act at both levels [7]. An inhibition of phospholipase by quercetin is also observed [66].

The various effects of flavonoids suggest that numerous pharmacological mechanisms are involved. One of these mechanisms involves an inhibition of phosphodiesterases hydrolyzing AMP_c and GMP_c; the concentration of GMP_c or AMP_c increases in various proportions depending on the flavonoid considered [92].

A structure-activity relationship can be identified for flavonoids; double bond in C_2 and C_3, hydroxyles on C_3, C_5, C_7, $C_{3'}$ or $C_{4'}$, aglycone structures are better inhibitors [14]. Other mechanisms through a cyclooxygenase or Ca^{2+}-ATP inhibition have also been suggested for catechin.

However this activity can be compared with that of ascorbic acid on the same molecules; enhancement of their effects not only by a direct action on adenylcyclase but also by inhibition of GMP_c and AMP_c phosphodiesterases at the end of hormone stimulation [68]. A similar comparison can be made between the hypocholesterolemia-promoting activity of some flavonoids, especially (−)epicatechin [6] and that of ascorbic acid, favoring the elimination of cholesterol as biliary acids probably at the level of the 7-hydroxylation of the steran ring level [54]. Thus, the various physiovascular activities of ascorbic acid and some flavonoids, in particular flavan-3-ols, do show a certain similarity with respect to their action on blood capillaries. However, there are numerous interactions between these compounds which must be considered.

Flavonoids-ascorbic acid relationships

The injection of natural and synthetic flavonoids improves the ascorbic acid level in different organs [81]. It has also been observed that plants with high ascorbic acid concentration have high levels of flavonoids. So a close relationship appears to exist between flavonoids and ascorbic acid, in the vegetable as well as the animal kingdom. Such interactions occur at various levels.

First, like tocopherols, flavonoids are antioxidants. Such compounds are most commonly found in food products and they interact either with hydrophilic or lipophilic systems. This antioxidant activity may be related to a minimal structure, about which different opinions have been expressed; it may involve hydroxyl groups on C_2 and C_3 or a 3-hydroxy 4-keto group. Two mechanisms may induce this behavior. Ascorbic acid is relatively stable in acidic pH solution but in neutral or alkaline solution its oxidation to dehydroascorbic acid is rapid. This oxidation which involves free radicals is catalyzed by Cu^{2+}. The antioxidant effect of flavonoids at low pH is likely to be of a free radical-trapping nature, whereas a metal-flavonoid complexation (especially with copper) occurs in neutral or alkaline media [100].

Several cuproproteic enzymes which bring about ascorbic acid oxidation (ascorbic acid oxidase, peroxidase) are also inhibited by flavonoids which thus intervene as factors which 'protect' or 'spare' ascorbic acid. This activity does not even appear to be an exclusively flavonoid-specific one. The vicarious action of B group vitamins, biotin and paraaminobenzoic acid, on vitamin C can also be regarded as an ascorbic acid protection.

However, some flavonoids have been observed to play a special role in dehydroascorbic acid reduction by thiols. This oxidoreduction reaction aims at maintaining the ascorbic acid concentration in the organs. The main pathway of ascorbic acid catabolism involves in the two first steps an oxidoreduction of ascorbic acid into dehydroascorbic acid, then the opening of the dehydroascorbic acid lactonic ring to form dicetogulonic acid. This last

oxidation is irreversible and leads to loss of physiological activity; dicetogulonic acid, unlike ascorbic acid or dehydroascorbic acid, does not have any antiscurvy activity[22]. The reversible oxidoreduction of ascorbic acid to dehydroascorbic acid appears to be a fast cell reaction which occurs in response to the organism's needs. It is dependent on the level of glutathione. The lactone ring opens when the concentration of glutathione in the organ is almost zero. The dehydroascorbic reduction by glutathione is catalyzed by flavonoid antiscurvy compounds[38] and this behavior has been shown not to be linked to the redox potentials. In fact, previously mentioned vitamins, as well as flavonoids which present an redox potential which makes them susceptible to involvement in dehydroascorbic acid reduction have no influence on this reaction, whereas flavan-3-ols in the monomeric or the natural form, catalyze this reaction[86]. Consequently, the antioxidant effect of flavonoids may imply two different types of activity. Along with a general activity, there is a more specific involvement of flavanol compounds.

A third kind of effect of flavonoids on ascorbic acid has also been demonstrated. This concerns gastrointestinal metabolism; an increase of ascorbic acid absorption in the presence of flavonoids has been shown, probably resulting from their antioxidant properties[28, 105]. Also, epicatechin has been proved to stimulate dehydroascorbic acid resorption by stabilizing it as ascorbic acid[108]. Nevertheless, the simultaneous injection of lemon flavonoids and ascorbic acid injection does not increase the concentration of dehydroascorbic acid in plasma[107]. From a more general point of view, a similarity exists between many of the metabolic targets of ascorbic acid and flavonoids; one example is cataract, in which both compounds intervene. This disorder in diabetic and galactosemic animals is characterized by a diminution or disappearance of ascorbic acid in the cristalline lens and in the aqueous humor and flavonoids inhibit the aldose reductase, a key enzyme of the opacification of the eye[76, 101]. Another example is leukemia, in which both ascorbic acid and flavonoids have been implicated[69]; the latter because of their reactivity with copper, which is an oligoelement and component of metalloproteins.

Therefore, besides various oxidoreduction phenomena in which ascorbic acid and flavonoids are involved, an examination of the physiological properties of flavonoids, including antiscurvy and vascular activities, as well as their protective action on ascorbic acid, has shown that there are more specific interactions between flavan-3-ols and ascorbic acid.

Distinguishing properties of flavan-3-ols

Among the whole range of flavonoids, flavan-3-ols present distinguishing properties at various levels as well as in the fields of physiovascular and antiscorbutic activity.

From a chemical point of view, these compounds are characterized by their hydrosolubility and their capacity to give a very reactive carbocation in an acidic medium as a consequence of the C_2 and C_4 electrophily[74]. It follows that flavan-3-ols are found in nature in the aglycone (and not in the heterosidic state) and that condensation reactions of nucleophilic substances, such as ascorbic acid, or dimerization and polymerization reactions can occur[53, 87, 102].

Together with these chemical arguments, metabolic and toxicological considerations ought to be advanced.

Not only because of their marked structural diversity but also because of the diversity of animal species which consume them, flavonoids present numerous metabolic pathways. Part of the diversity of these pathways results from the fact that they are also metabolized by various organisms of the gastrointestinal microflora in the digestive tracts of the different animal species. Very complete reviews have recently been written on this topic[49, 51]. However, in comparison with other flavonoids, flavan-3-ols show some characteristic metabolic pathways: the main pathway involves a methylation to give 3 or 3'-methyl esters, and the intestinal absorption, in contrast to that of quercetin and other flavonoids, is high. (^{14}C) catechin administration gives a strong radioactivity especially in adrenals but also in muscles, liver, spleen and kidneys, and even, in high concentrations, in the glycosaminoglycans of connective tissue such as skin, aorta, trachea and cartilages.

From a toxicological point of view, in the last few years, some flavonoids have been noticed to have mutagenic properties. For a long time flavonoids were considered as non-toxic substances but their effects on enzymes, some of which are involved in the formation of carcinogenic metabolites, show evidence of their action at the genetic level. The Ames test[1] revealed that flavonoids with the following structures were mutagenic; a free hydroxyl in position 3, an unsaturated bond in the 2–3, ketogroup in 4 and a structure allowing protonation of hydroxyls in 3 and keto-3 group tautomerization[99]. Some flavones which are not hydroxylated in position 3 also show a mutagenic effect on bacteria. This effect is induced by aglycones, but glycosidic associations, while not mutagenic in themselves, are potential pro-mutagens; they release aglycone in the presence of a suitable glucosidase (from the intestinal flora, for example). Numerous flavonoids (flavones, flavanones etc.) and especially flavonols present mutagenic properties: quercetin and kempferol are the most potent flavonoid mutagens. It has also been suggested that quercetin may be an incomplete carcinogen or initiator but this question is presently being debated[24, 72]. Consequently, the ubiquity of flavonoids in the majority of food plants may be dangerous. However, the low quercetin absorption in the gastro-intestinal tract (less than 1% can be absorbed without being modified) limits its potential toxicity. At the opposite extreme, the flavan-3-ols have neither the type of structure related to mutagenic properties, nor are they

metabolized in the gastrointestinal tract so as to produce such structures and, so far, there are no reports of these compounds being toxic. Flavan-3-ols have been submitted, before and after metabolization, to the Ames test and show a complete absence of any mutagenicity [74].

So, besides their characteristic chemical reactivity and physiological properties with respect to scurvy and blood vessels, flavan-3-ols are characterized by their 'bioavailability and innocuousness' as Masquelier et al. [74] emphasize. That is the reason why they are different from other flavonoids and must be distinguished from the whole range of flavonoids and polyphenols.

Conclusion

After the consideration of these various arguments, is it possible to answer the questions set out in the introduction?

First of all, the 'Vitamin P' notion is obviously different from Szent Györgyi's definition. Nowadays, it refers to a pharmacological effect which is not specific for substances classified as 'Vitamin P-like'. The bioflavonoid concept, which underlines the ubiquity and basic character of flavonoids, is no longer adequate because whereas some flavonoids have been found to have a mutagenic effect, others present no toxicity and have a beneficial physiological effect on mesenchyme tissues. Some authors, 'mostly botanists', emphasize the structural unity of these compounds and propose the nomenclature 'pycnogenols' which means 'tannin-generators' [74]. However this term, which is fully justified in plant taxonomy, does not underline the important role played by flavan-3-ols in animals, especially their nutritional function as an antiscurvy factor for men and species susceptible to scurvy. This activity only occurs in the presence of ascorbic acid; therefore neither ascorbic acid, nor the flavan-3-ols can be described as 'the antiscurvy vitamin', but the association of both of them, at a low dosage, has the same effects as an antiscurvy vitamin would. In this context, flavan-3-ols appear to be a second antiscurvy factor which is necessary but not sufficient for preventing or curing scurvy, since it is inactive without ascorbic acid. Inasmuch as flavan-3-ols exert their own action on ascorbic acid, and their cellular targets support the effects of ascorbic acid, these compounds can be considered as 'a protecting' or 'sparing' factor for ascorbic acid. They are essential for the correct functioning of organisms susceptible to developing scurvy. That is why the notion of a necessary but not sufficient, second antiscurvy factor seems to be appropriate.

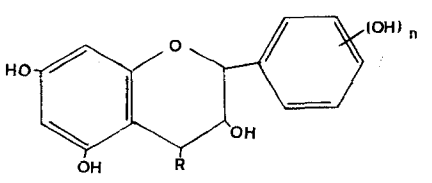


Figure 3. Generic structure of C_2 factor. n = 2 (catechin) or 3 (gallocatechin); R = H (flavan-3-ol), = OH (flavan-3,4-diol); ? flavan-3-ol (proanthocyanidin: with n = 2 procyanidin, with n = 3 prodelphinidin).

This concept must be discussed in detail. The physiological properties of flavan-3-ols and the pharmacological and toxicological arguments above, allow us to consider that the C_2 factor concept corresponds to a generic notion characterized by the following minimal structure; a phenylchromane structure hydroxylated at positions 3, 5 and 7 and with a polyhydroxylated phenyl ring in position 2 (fig. 3). A substitution by a hydroxyl in position 4 (and the flavan-3-ol structure becomes a flavan-3,4-diol) or by another flavan-3-ol molecule to give a proanthocyanidin dimer may also be considered. So, as with vitamins A, D, or K, the notion of the C_2 factor includes several compounds whose degree of efficiency should be enhanced with a cis-isomerism in position 3 or a higher number of hydroxyl groups on ring B. The comparison with the above vitamins may be extended to another concept, the antivitamin notion, since these compounds can form high polymers which show scurvy generating properties.

Thus the chemical and physiological unity of the group of flavan-3-ols as antiscurvy compounds can be contrasted with the diversity of substances classified as 'Vitamin P' or as 'Bioflavonoids'. In order to recognize the far from negligible physiological and nutritional role of these compounds, the nomenclature proposed by Randoin et al., in the twenties, i.e. 'second antiscurvy factor' or 'C_2 factor', ought to be used.

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