

NATURALLY OCCURRING FOOD TOXICANTS: PHENOLIC SUBSTANCES OF PLANT ORIGIN COMMON IN FOODS

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I. INTRODUCTION

The risks to humans of serious poisonous effects caused by natural phenols present in normal foods consumed under usual circumstances seem vanishingly

small, as determined by experiment as well as by common sense and long-term observation. Why, then, consider the matter further? Phenolic substances are naturally present in essentially all plant material, including food products of plant origin, and sometimes in sizable amounts. A few phenols occurring in nature are potent toxins; some have caused serious outbreaks of toxic dietary reactions in animals. In a few instances, deleterious effects in humans appear to be the result of an abnormal consumption of plant phenols or consumption of phenols abnormal to the diet. On the basis of tests with experimental animals, phenolic derivatives found to be carcinogenic or otherwise seriously toxic at any level—safrole and coumarin, for example—have been banned as food additives. Common flavonols recently have been found to be bacterial mutagens. Public agencies are demanding extensive testing if not summary elimination from food of any molecule that is not necessary for life. Phenols, with a few specific exceptions, are considered to be secondary metabolites (nonessential for life), even in plants.

On the other hand, some kinds of plant phenols appear to have desirable physiological effects in diets or as drugs. Processing advantages, such as the antioxidant activity of certain phenols, can be important not only in maintaining a continuity of supply of nutritious and inexpensive food, but also in providing a protective effect against photocarcinogenesis through lipid oxidation (Logani and Davies, 1980).

In many countries, particularly the economically disadvantaged ones, the diet is almost exclusively vegetarian, with a consequent increase in plant phenol intake. The current enthusiasm for vegetarian diets in the United States is partly a fad, but economics and population pressures are likely to reinforce the trend. The phenol content will increase in proportion to the substitution of plant for animal products in the diet. If exotic plant products or additional contaminants are taken into the diet as a result of emergencies or other factors, unusual phenols may be introduced as well—gossypol or aflatoxins, for example. Singleton and Kratzer (1973) made a case for the idea that common plant phenols are nontoxic only because of efficient animal metabolism of them under normal circumstances.

In view of these considerations, an objective sought in this review is to place the common phenolic substances of the plant kingdom in perspective as real or potentially toxic or physiologically active substances in human and animal diets. An attempt will be made to focus on the compounds as phenols or derivatives of phenols rather than the more usual practice of singling out only certain classes of phenols (e.g., anthraquinones, coumarins, flavonoids, lignins, tannins), certain effects (e.g., carcinogen, estrogen, photosensitizer, vitamin antagonist), or certain sources (e.g., angiosperms, fruits, grains, legumes, mycotoxins).

Absolute completeness is impossible and coherent synthesis is difficult because of the voluminous, disciplinarily diverse, and scattered literature from the nutritional, medical, pharmacological, physiological, toxicological, biochemical, chemical, and agricultural fields that relate to the topic. No individual, it is

freely acknowledged, can be thoroughly expert and up-to-date in all these interrelationships. The information to be presented has been drawn from participation in cooperative research on inhibition by natural phenols of growth in chicks (Kratzer *et al.*, 1975) and in the preparation of two reviews (Singleton and Kratzer, 1969, 1973). Literature searching was based on that done for the previous research and on reviews plus references obtained in an effort to remain currently aware of developments in the field. More intensive manual searching of *Chemical Abstracts* in 1977 and 1978 was supplemented by a computerized search of *Biological Abstracts* (BIOSIS) files for specific phenol-animal interactions reported in reviews since 1969 and in individual papers from 1977 to October 1980. About 2000 papers were read or scanned and their bibliographies compared in an effort to include all important references. The references cited are limited, generally, to the most recent reviews or papers. Further references may be obtained from these cited articles by those desiring more background.

Much of the discussion will be on specific examples of the toxicity of common phenols of plant origin. It is important to note, however, that toxicity and favorable therapeutic effects can overlap, and the way a compound is viewed may depend on how it was first discovered and tested. For example, dicoumarol was discovered as the cause of a bleeding death in cattle, and the discovery led to a useful rat poison. Development of dicoumarol and its derivatives as useful drugs to prevent unwanted clotting in cases of threatened embolisms and strokes was somewhat impeded by the negative image. In other cases, useful therapeutic activity was discovered first and serious toxicity later, with the result that usage has been tolerated more than in the reverse situation (diethylstilbestrol may be an example). Therefore, physiological effects as well as toxicity will be considered.

II. ORIGINS AND TYPES OF PLANT PHENOLS

The phenols of nature are mostly biosynthesized via one of two major pathways or their combination (Hahlbrock and Grisebach, 1975; Harborne, 1980). One mechanism typically produces *m*-trihydroxyphenols resembling phloroglucinol by cyclization of polyketides derived from linking at least three two-carbon units equivalent to activated acetate. The second mechanism synthesizes phenols via the shikimic acid pathway to phenylalanine and thence to C₆-C₃ phenols such as *p*-hydroxycinnamic acid and tyrosine. Phenols from this mechanism typically have an hydroxyl para to the three-carbon side chain and often have additional hydroxyl groups or derivatives thereof adjacent to the first. Flavonoids are produced by a combination of the two mechanisms so that the A-ring is "acetate"-derived and has meta-hydroxylation and the B-ring is shikimate-derived with 4' or more hydroxylation. Typical flavonoids are malvidin, an anthocyanin, and quercetin, a flavonol (Fig. 1). Other biosynthetic path-

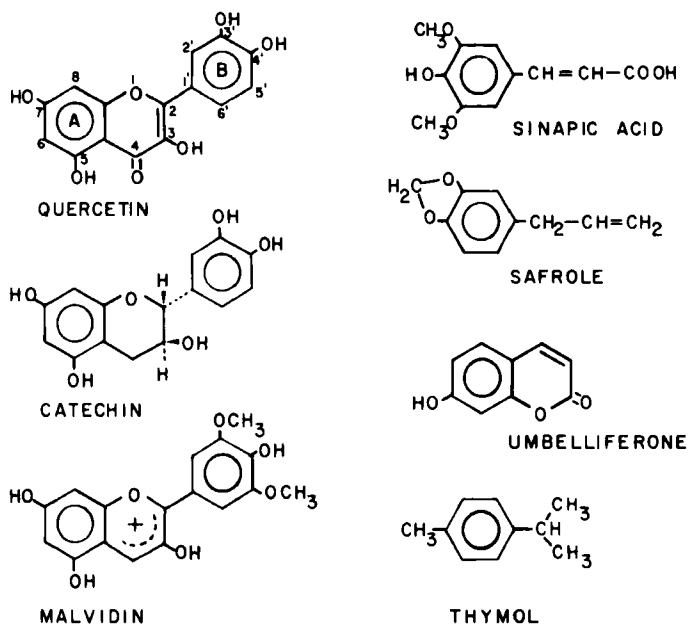


FIG. 1. Examples of phenols common and uncommon in foods from plants: common—quercetin (flavonol), catechin (flavan-3-ol), malvidin (anthocyanidin), sinapic acid (hydroxycinnamic acid); uncommon—safrole (4-allylphenol, methylenedioxy ether), umbelliferone (coumarin), and thymol (terpenoid phenol).

ways modify the phenols produced and in some instances such as certain terpenoid phenols, create other less common types of phenols. Terpenoid phenols like thymol can usually be recognized by their isoprenoid subunits.

Flavonoids (anthocyanins, flavan-3-ols, flavones, flavonols, etc.) differ by the degree of oxidation of the heterocyclic ring formed from the three-carbon side chain of the phenylalanine part and one hydroxyl and two carbons of the “phloroglucinol” part. Chalcone, aurone, isoflavonoid, and neoflavonoid derivatives are generally included along with the flavonoids, owing to their similar origins and structures.

Coumarin is considered a shikimate-derived phenol, since it is the lactone of 2-hydroxycinnamic acid (*o*-coumaric acid). Similarly, methoxy and methylenedioxy derivatives (e.g., safrole, Fig. 1) and any other linkage that is clearly biochemically or chemically derived from phenols—quinones, for example—are commonly included as phenols. A few exemplary structures are given in Fig. 1. Sinapic acid (a cinnamic acid), safrole, and umbelliferone (a coumarin) are shikimic derivatives. Quercetin and (+)-catechin (a flavan-3-ol) are probably the two flavonoids most widespread in nature. The structures of

most other natural phenols are readily available from many good books and reviews (Harborne, 1975, 1980; Anonymous, 1976; Dean, 1963; Robinson, 1980).

Further important natural phenols include lignans and lignin, which are dimers and polymers, respectively, of C_6-C_3 units of the hydroxycinnamate type. Tannins are large polyphenolic molecules that are either condensed tannins (dimers and larger of flavan-3-ols, commonly) or hydrolyzable tannins (commonly glucose or quinic acid esterified with gallic acid, 3-digallic acid, or hexahydroxydiphenic acid). When released by hydrolysis, hexahydroxydiphenic acid (a dimer of gallic acid) forms the dilactone ellagic acid; hence the hydrolyzable tannins are also called gallotannins or ellagitannins.

For the purposes of our discussion alkaloids are excluded, even though many of them are phenolic as well as nitrogen-containing (e.g., capsaicin, colchicine, mescaline, morphine).

Each of these general types of natural phenols can be subdivided into members of common, uncommon, or botanically rare occurrence. Most of the phenols noted for toxicity or physiological potency in animals not only are extremely limited in botanical distribution, but also are unique or highly unusual in their chemical structure when compared with common plant phenols (Singleton and Kratzer, 1973).

III. EVOLUTIONARY CONSIDERATIONS

Conclusions involving evolution obviously are speculative, but some putative relationships appear worthy of discussion to clarify the durability of animal life and the threat of plant toxins generally or of phenols particularly. Most of the food plants of agriculture are highly evolved and highly selected angiosperms. The common phenols within this restricted group include a few dozen substances. The total amounts of these common phenols can range from nearly zero in refined, plant-derived foods like table sugar, to 20% or so of the dry weight of the diet of some herbivorous animals.

Given a free choice, animals in general and man in particular select food plant parts with a low phenol content from a lignin (toughness) or tannin (astringent taste) viewpoint, but perhaps a relatively high anthocyanin content (visibility, ripeness indicator). Particularly with agriculture, man has not only selected and modified by breeding the plants he cultivates for feed and food, but in a great part of the most fertile land he has completely changed the "ecology" in terms of the relative diversity and "commonness" of individual plant species and the resultant occurrence of various phenols. We therefore may have a warped and limited view of the possible risk of plant toxins. When domesticated animals were introduced by Europeans to unfamiliar flora in Australia, about one plant species

in twenty was found capable of causing death (Culvenor, 1970). The ratio is a little more favorable in native North American or African flora, but if we allow for unrecognized, slow-developing, or subacute toxicity, it is quite clear that many plants are not entirely benign to animals. With the apparent exception of the grasses and grains of the Gramineae, few plants have adapted specifically to allow predation by animals without developing some form of active resistance, such as thorns or toxins (Culvenor, 1970). Fruit may be sacrificed to an animal in return for seed dispersal, but often the seeds themselves may be toxic or may at least resist digestion. Many plants have evidently evolved toxins to help protect them from pests, parasites, disease organisms, or predators. Animals have evidently evolved and survived by "learning" to avoid or withstand these toxins.

A. PHENOLS FROM PLANTS

Phenols are probably the most important group of substances useful in chemotaxonomic differentiation among plant species (Harborne, 1975). The reasons for this include the fact that some phenols are found in nearly all plants, but the qualitative diversity among different plants is large. Not counting diversity caused by variation in the formation of derivatives, especially in glycosidation, at least 800 different phenolic aglycones from plants were known in 1968 (Singleton and Kratzer, 1969), and owing to a continuing rapid rate of discovery there are probably triple that number today (Harborne, 1979, 1980). For taxonomy, phenols of limited distribution are, of course, more useful than those common to most plants. A phenolic constituent unique to a single species is also not a very useful taxonomic key, but searching for it in related plants may show it or related compounds to be more widely distributed than was at first supposed. Although chemotaxonomy is still a developing field, considerable useful information is available from it and from chemical studies on lichen products, mold products, antibiotics, unusual plants, etc.

In the most primitive plants, flavonoids are essentially absent, and the phenols of molds and lichens are mainly "acetate"-derived polyketides with meta-hydroxylation. They include, nevertheless, a large and diverse group of structures. Flavonoids are found in mosses, ferns, and gymnosperms—but generally in limited and less usual forms (Swain, 1975). They become more common and more diversified in angiosperms of increasingly evolved forms. Trees and vines, however, generally have a higher content and more different (but common) phenols than do the still more highly evolved herbaceous plants.

The common phenols of the agriculturally predominant angiosperms include the cinnamic acid derivatives caffeic, *p*-coumaric, ferulic, and sinapic acids (respectively, 3,4-dihydroxy-, 4-hydroxy-, 3-methoxy-4-hydroxy-, and 3,5-dimethoxy-4-hydroxycinnamic acids). These cinnamates usually occur as esters of quinic acid (chlorogenic acid and analogs) or other nonphenolic hydroxyl

compounds (glucose, shikimic acid, tartaric acid, etc.). Gallic acid (3,4,5-trihydroxybenzoic acid) is widely found in plants, and gallotannins and ellagitannins also are not uncommon, especially in certain woody species. Lignin is nearly universal in higher plants, although the level may be high or low, depending on woody versus herbaceous habit. Lignin precursors such as coniferyl alcohol and lignin fragments are also widespread, but usually low in amount.

Certain flavonoids are common. The most primitive plants with flavonoids tend to lack 3-hydroxylation, but the usual pattern in higher plants is based on the 3,5,7-trihydroxyflavan structure. The common natural anthocyanins are glycosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. The B-ring hydroxylation pattern is like that of the cinnamates or gallic acid. Other flavonoids considered very common in plants include the flavones apigenin and luteolin, the flavonols kaempferol and quercetin, the flavan-3-ols (+)-catechin and (–)-epicatechin, the flavanone naringenin, and the flavanoneol taxifolin. The condensed or flavonoid tannins are dimers and larger polymers of the catechins. They are often called anthocyanogens, proanthocyanidins, or polymeric leucoanthocyanidins because when properly treated they usually produce cyanidin. They occur very widely, especially in woody plants.

The common phenols, then, in terms of their consumption by animals, include a few dozen substances. Other phenolic derivatives—coumarins, for example—and other substitution patterns are not necessarily rare, but they are less common, particularly in the usual unrestricted diet of animals and man. The remaining 90% or so of the known natural phenols are relatively rare among plant species and in animal diets.

The functions of phenols in plants have been a continuing puzzle, but it is clear that one function can be protection via toxicity. Certain substances—many of the identified ones are phenols—called phytoalexins are produced in plant tissue upon injury by pathogens and are sufficiently inhibitory to the invader to produce resistance (Deverall, 1972; Kuc, 1976). Such inhibitory compounds can have a toxic effect in animals too. A series of furane derivatives (not phenolic) that are produced as phytoalexins by sweet potato tissue after attack by certain molds, yeasts, or bacteria have figured in repeated episodes of poisoning of farm animals (Wilson and Hayes, 1973).

The general response to wounding and recovery in plants commonly involves production of a high level of phenols adjacent to the injury, along with oxidation of the phenols by polyphenoloxidase to quinones and phytomelanin brown polymer complexes. The oxidation products are often more toxic to potential invaders than are the original phenols, and the polymerization helps seal off the injured surface and begin the healing process. In some instances unusual phenols and other toxic compounds are clearly part of the normal resistance mechanism of a plant. Gossypol appears to confer resistance in cotton to some pests, for

example, but specialist predatory insects often evolve to tolerate the toxins of a certain plant. Phenols even appear to be involved in hypersensitive self-toxicity reactions within a given plant and in allelopathic competition among plants (Muller and Chou, 1972).

Summaries indicate the importance of phytotoxins, some phenolic, in the defense set up by plants against herbivores (Feeny, 1976; Harborne, 1977; Rhodes and Cates, 1976; Rosenthal and Jansen, 1979). Two classes of phytotoxins seem employed to combat herbivores. One class includes the generally and weakly inhibitory substances, notably tannin, which in larger amounts decreases the digestibility of the plant proteins or otherwise interferes with their consumption, owing to its disagreeable taste or other effects. The second group is more specifically toxic and requires less "investment" of photosynthate. Some plants in nature are readily found by herbivores (including insects) because they are conspicuous and long-lived. An oak tree, for example, is apt to depend on a high tannin level (and high lignification, etc.) to defend itself against all comers and to make a large biosynthetic investment in such defense. Such plants may also make specific toxins of higher potency. An ephemeral plant, inconspicuous and short-lived, is more likely to escape specialist predators and is not apt to make such a heavy investment of photosynthate in toxins. If toxins are made, they are usually "intended" for the generalist herbivore, but they are also likely to be more immediately toxic or repellent in low concentrations. Immediate cessation of feeding is most effective for plant survival, although a slowly acting poison eventually can be effective if it suppresses the predator population.

Schoonhoven (1972) visualizes an evolutionary sequence resulting in an obligate association between certain insect herbivores (and their predators) and certain plants. A plant produces a new deterrent substance. An insect adapts to overcome the deterrent, thus avoiding competition. The specific plant's unusual composition becomes an attractant, a feeding stimulant, and in some cases a requirement for such functions as oviposition. Ultimately in stepwise coevolution, an enormous diversification and specialization developed among plants and herbivorous insects. Apparently, the diversity that makes plant phenols especially useful in chemotaxonomy of species and races has, at least partly, resulted from efforts to escape phytophagy (Harborne, 1978). By agriculture's homogeneous concentrations of relatively benign plants, man is inadvertently encouraging local population explosions of both specialist and generalist insect herbivores.

There are a number of clear-cut examples of phenolic involvement in insect specialization (Schoonhoven, 1972; Harborne, 1979). Larvae of *Tortrix viridana* inactivate oak leaf tannin by binding it in special cells in the gut wall. The silkworm, *Bombyx mori*, is attracted to and stimulated to feed by the two major flavonoids, morin and quercetin-3-glucoside, of its host, mulberry, *Morus alba*. In fact, mulberry phenols are required as dietary constituents for proper development of the insect (Kato, 1978). Furthermore, other flavonoids with rela-

tively small structural differences, such as quercetin-3-rhamnoside, will trigger avoidance and stop feeding (Schoonhoven, 1972; Harborne, 1979). Armyworms capable of tolerating 0.1% umbelliferone in the diet were killed by 0.1% xanthotoxin, a furanocoumarin biosynthesized from umbelliferone. This is an example of how plants escape from a generalist insect herbivore by producing a new secondary product (Berenbaum, 1978).

An unusual combination of common plant phenols, quercetin-3-(2''-galloyl)glucoside, produced 100% mortality in 12 hr in three species of snail fed at 10 ppm in leaves (Dossaji and Kubo, 1980). Examples of plants combating higher animals feeding on them are not so specific. Many studies with domestic animals and some with wild browsers show that high-tannin material is avoided. Milton (1979) found that herbivorous howler monkeys selected leaves with the highest protein and lowest fiber (lignin included) content available. Tannin or total phenol content appeared to affect choices among plant species, but not within species.

B. ANIMAL ADAPTATION

Plants have evolved toxins to combat herbivores, but herbivores have evolved also to tolerate or avoid plant toxins. When a new toxin is initially contacted in small amounts, animal metabolism can often adapt in order to detoxify it in subsequent higher exposure (Feeny, 1976). The astringency of tannins and the bitterness of some phenols (and alkaloids, glycosides, etc.) are repellent to animals, at least at high intensity (Bate-Smith, 1972). The loss of tannin in the evolution of related plants is often associated with the development of alkaloids or other toxins or repellents. Animals differ by species, breed, and individual in food selection in relation to phenol content (Arnold and Hill, 1972). Coumarin, for example, is offensive to sheep (Harborne, 1977) and quite toxic (Singleton and Kratzer, 1969), whereas its odor is considered attractive to humans. Thus, it is quite clear that animals do adapt to phytotoxins and evolve differently, depending on exposure.

Carnivores have the least exposure to plant phenols. Consequently, there appears to be a great toxicity of phenols in strict carnivores (and insectivores), less in omnivores, and least in strict herbivores (Singleton and Kratzer, 1969). That this is not due to the habitual microbial flora of the digestive tract, or at least not entirely, is indicated by similar relative toxicities observed after both parenteral and oral administration in the different species. Comparisons of the LD₅₀ values collected from numerous sources so that such conclusions could be drawn are subject to many valid criticisms, not the least of which is the paucity of examples of the same phenolic preparation tested in a diverse range of animal species under comparable conditions. There are few species of animals that are inexpensive and convenient enough for extensive acute or chronic lethal toxicity studies to be made. Nevertheless, it is some comfort to humans that the higher

primates are primarily vegetarians (herbivores) by nature, and man is the omnivore par excellence, with an herbivore-scavenger background only recently (geologically speaking) and regionally heavily carnivorous. Humans, therefore, should be moderately resistant to plant phenols and other phytotoxins—and this appears generally to be true.

Furthermore, mammals in general seem to be adapted to angiosperms as their diet. Angiosperms gained ascendancy over gymnosperms and more primitive plants at about the same time that mammals were replacing reptilians. An interesting postulate is that the phytotoxins of the angiosperms were the reason or an additional reason for the demise of the dinosaurs (Swain, 1974; Rosenthal and Janzen, 1979). It is an intriguing idea, one that appears to be buttressed by the fact that the surviving reptilian species are seldom plant eaters. Fish and frogs tend to be quite sensitive to plant phenolic toxins (rotenoid fish poisons, gossypol in trout, and low LD₅₀ values for phenolic toxins in frogs come to mind). The high toxicity of aflatoxins to birds may be related to their reptilian connection.

On the other hand, the surviving lichens, ferns, and gymnosperms often are quite toxic to mammals, and some of the toxins are phenols of unusual types. Perhaps the plants surviving from the primitive groups were the more toxic members, and the metabolism that enabled mammals to combat angiosperm phytotoxins was less successful. The microorganisms, which are primitive but highly adaptable forms, also remain, in some instances, as the producers of very potent toxins to animals, some phenolic.

Arthropods, especially insects, sometimes sequester plant toxins and use them in their own defense (Rodriquez and Levin, 1976). The best known examples, such as the milkweed cardenolides in the monarch butterfly, are not phenols, but a few examples are phenolic or related, such as the accumulation of phenol, hydroquinone, juglone, salicylaldehyde, hypericin, or mellein by various specialist insect herbivores (Rodriquez and Levin, 1976; Schoonhoven, 1972). Insects even make use of the "plant" resistance mechanism of phenol oxidation, polymerization, and tanning to harden and darken their "skin," to inhibit pathogens and to seal lesions (Taylor, 1969). Again illustrating the evolutionary development of interacting toxins, some phenols from certain plants have a hormonal effect on insect feeders that forces them to remain juvenile (sesamin, a lignan from sesame) (Singleton and Kratzer, 1969) or converts them prematurely to adult forms (precocenes, chromenes from *Ageratum houstonianum*) (Ohta *et al.*, 1977).

C. ANIMAL METABOLISM OF PHENOLS

Higher animals cannot make compounds with benzenoid rings from aliphatic precursors, with very few exceptions (estrone and related phenolic steroids being one). Plants are the source of nearly all the phenols found in animals. Even the

essential phenols for animals (tyrosine, catecholamines, vitamin E tocopherols, vitamin K menadiones, thyroxine) are drawn either directly or indirectly from plants or are modified from an essential plant precursor, usually phenylalanine. Tyrosinase, which is common to animals, is very similar to plant phenolases. Oxidation of phenylalanine to tyrosine and insertion of a second phenolic hydroxyl to form dopa, followed by oxidation to quinone derivatives and polymerization to melanins and melanoproteins or related adducts, show a facile management of phenolic compounds by animals.

It is clear that animals are accustomed to efficient and rapid removal, "detoxication," of excess phenols of these types, which are so potently active in the animal body. Control of nerve action depends on rapid removal of norepinephrine, for example, immediately after it has performed its nerve transmission function. If excess norepinephrine is injected, it is very toxic. Benzenoid compounds, not themselves phenols, are commonly hydroxylated in the animal body as an early step in their catabolism—phenylalanine, for example. Foreign (xenobiotic) phenols taken in by animals are metabolized and detoxified by mechanisms similar to those used for normal animal phenols. The toxicity of a given xenobiotic phenol in an animal involves conditions or structures that are unsatisfactorily managed by the animal's detoxication system or that overload the system's capacity.

It is possible for "detoxification" to convert an innocuous compound into a toxic derivative. An example is seen in hydrocarbon carcinogens such as benzpyrene. The hydrocarbon itself is not toxic or carcinogenic, but a series of derivatives are produced in the body, some of which are toxic and carcinogenic. The derivatives are produced by enzymic epoxidation of certain double bonds followed by hydration of the epoxide to a *trans*-dihydrodiol and further dehydration to a phenol or oxidation to quinones (Heidelberger, 1972). Metabolic activation to a reactive electrophile is involved in producing the carcinogenicity (Cohen and Moore, 1977). However, of the at least five phenols, three quinones, three diols, several epoxides, and various conjugates produced (Yang *et al.*, 1977), only a few are appreciably carcinogenic in various tests. Several phenolic derivatives were non-carcinogenic, but 2-hydroxybenz(*a*)-pyrene was highly carcinogenic on mouse skin (Wislocki *et al.*, 1977). Quinoidal derivatives produced would also be toxic because of the continuous production of hydrogen peroxide and interference with normal oxidation-reduction systems (Lorentzen and Tso, 1977). Thus, detoxication and assessment of the active form of a toxicant can be complicated, and phenols may be involved in less obvious ways.

The liver is considered the primary site of enzymic detoxication in animals, although the intestinal mucosa (Shirkey *et al.*, 1979; Stohs *et al.*, 1977), renal tubules (Fry *et al.*, 1978), lung, skin, and placenta (Pelkonen and Moilanen, 1979), and perhaps other tissues can be involved as well as nonspecific reactions and "external" factors, such as the microflora of the alimentary canal. The

majority of plant phenols, like other xenobiotics, are metabolized in two phases: (I) conversion of the basic structure into a new form, usually more oxidized but sometimes reduced or hydrolyzed, and (II) conjugation with some natural constituent from the body. The conjugates are then excreted in the urine or the bile. Once conjugated, the product is invariably nontoxic, or at least much less toxic than before conjugation. Any one phenol may be incompletely absorbed (if orally introduced), excreted partially unchanged, or excreted partially in the form of one or more metabolites without conjugation; the rest is excreted with conjugation, with or without initial metabolism. Most substances produce more than one excretory product, and some produce many; therefore, a total inventory of the amount administered is frequently difficult.

Other tissues may be important in conjugation, but the phase I metabolism is performed mostly in the liver. When the cells are experimentally disrupted, the endoplasmic reticulum membrane in the liver cells forms tiny sacs called microsomes, which have detoxication capabilities. The enzymes involved in detoxication are of low specificity, but they are highly inducible by exposure to a new foreign compound to increase greatly both in specific ability to metabolize that substance and in the amount of enzyme (the rate of processing) available for the task. Thus, preliminary or chronic exposure may raise the minimum toxic dose of a drug or toxin considerably. The fetus or infant is particularly vulnerable to drugs and toxins, owing to the late development and low initial adaptation of this system.

The main enzymes involved in phase I are the liver microsomal mixed-function oxidases. Considerable detail is known about these detoxication mechanisms. Useful references include Jenner and Testa (1978), Kappas and Alvares (1975), Parke and Smith (1977), Testa and Jenner (1976), and Williams (1959). Molecular oxygen is required, as is reduced nicotinamide adenine dinucleotide phosphate (NADPH). Cytochrome P-450 serves as the terminal oxidase. It is believed to function by (a) binding the foreign substance, (b) being reduced from the ferric to the ferrous form (in the cytochrome heme) by interaction with NADPH and cytochrome P-450 reductase, (c) accepting O₂, and then (d) liberating the oxidized metabolite (one oxygen per molecule) of the foreign molecule with the ferric heme restored and the second oxygen atom, generally, appearing as water. Specific detoxications apparently may induce differences. The aryl hydrocarbon hydroxylase affecting benz(a)pyrene, for example, uses cytochrome P-448 rather than P-450 (448 nm absorbance maximum of the carbon monoxide adduct) (Kappas and Alvares, 1975). Owing to the low specificity of these enzymes, induction by one foreign substance of efficient detoxication may serve as a protection against additional foreign substances not previously encountered by the animal. On the other hand, there is a finite capacity to adapt to a load of substances requiring detoxication, and an additional foreign substance may interfere with detoxication or may increase the toxicity of another.

There are many conjugation reactions (Williams, 1967), and additional ones are still being discovered (Jenner and Testa, 1978). Those considered usually important in detoxicating phenols are methylation and conjugation with glucuronic acid, sulfate, or (for phenolic acids) glycine (hippuric acid formation). Conjugation of sulfate with a phenol to give the so-called ethereal sulfate (ester of sulfuric acid) is produced by means of activated sulfate, 3'-phosphoadenosine-5'-phosphosulfate (PAPS), and sulfokinase (Dodgson, 1977). The PAPS is formed by reactions consuming two units of ATP with the resultant loss of useful energy from other reactions. Glucuronic acid conjugation (Dutton, 1966, 1978; Williams, 1967) costs not only the energy necessary to produce the active transfer agent uridine diphosphoglucuronic acid (UDPGA), but also the potential energy and substance of the glucuronic acid. Similarly, an activated methyl group in the form of *S*-adenosylmethionine and the ATP consumed in its formation are lost from "normal" metabolism, if they are consumed in detoxication. Glycine or glutamine conjugation would sacrifice the respective amino acids. In animals on a marginal diet, these costs of detoxication may be the difference between barely normal and abnormal health.

No experimental animal adequately parallels man to serve as a satisfactory substitute in estimation of toxicity. Predictive values for toxicity in man have become more rather than less obscure as toxicity tests have become more detailed and have been done with more test species (Hayes, 1970). Qualitatively, the detoxication of a given substance by different animals may be similar, but quantitatively there are likely to be large differences not only among species, but among individuals of the same species under different conditions (Davies, 1977; Truhaut, 1978). The animal's age, sex, diet, and environment affect toxicity and detoxication. Even the route of administration may be significant; in a study with hens, intramuscular injection of phenol gave 20:1 sulfate to glucuronoside, whereas oral and intraperitoneal administration gave less than 3:1 (Capel *et al.*, 1974).

In an example illustrating species differences, phenol's urinary metabolites were phenyl sulfate, phenyl glucuronoside, quinol sulfate, and quinol glucuronoside in man and the mouse, rat, guinea pig, jerboa, gerbil, hamster, and lemming, but only the phenyl glucuronoside was detected in the pig and the two sulfates in the cat (Capel *et al.*, 1972). The urine of the dog, rabbit, ferret, and hedgehog lacked the quinol glucuronoside, and that of the squirrel monkey and capuchin lacked the quinol sulfate. About 12% of the urinary metabolites in sheep was phenyl phosphate (Kao *et al.*, 1979). The cat's very weak ability to make glucuronic acid conjugates may evolutionarily reflect its carnivorous habit (Capel *et al.*, 1972; Williams, 1967). The sulfate conjugation appears to be evolutionarily older than the glucuronide conjugation. Fish, aquatic turtles, and amphibia are usually weak in liver detoxication enzymes (Williams, 1967). High glucuronide conjugation relative to sulfate appears to be characteristic in the pig,

a monogastric near-vegetarian, and it is also characteristic of New World monkeys. Old World monkeys and man, however, are highly capable in both sulfate and glucuronic acid conjugation (Smith and Caldwell, 1977). Nonprimates and prosimians convert arylacetic acids to glycine conjugates, but monkeys, apes, and man make glutamine conjugates of these compounds.

Unlike the young of most laboratory animals, the human fetus and newborn infant are usually able to oxidize and detoxify a number of drug substrates. This ability seems not to be a result of exposure *in utero*, although that can have a role as well (Davies, 1977). It seems fair to conclude that man is relatively capable among animals of metabolically coping with ingested plant phenols and xenobiotics generally.

The dose affects the pattern of conjugation. The capacity to conjugate sulfate is generally limited, and therefore, for man and other animals capable of both sulfate and glucuronic acid conjugation, glucuronosides become a higher proportion of the excretory products with higher dosage (Mehta *et al.*, 1978). Excretion of a relatively high portion of administered phenol as quinol sulfate in the cat is considered an indicator of slow conjugation and excretion, resulting in a longer residence time in the body and more metabolism of phenol to quinol (Mehta *et al.*, 1978).

Detoxication is not complete until the substance is eliminated from the body. Excretion in the urine is the primary route for elimination of most water-soluble substances and conjugates. However, elimination via the bile can also be important (Griffiths and Barrow, 1972b; Hackett *et al.*, 1979; Smith, 1973). Since the bile empties back into the intestine, enterohepatic recirculation of the substance usually occurs, with resultant slower elimination in the feces. The conjugate groups in bile excreta may be hydrolyzed off by intestinal microflora and allow reabsorption of the more toxic form. In general, the larger molecules are excreted in the bile (Hirom *et al.*, 1976). In the molecular weight range of 350–450, there is usually extensive elimination in both bile and urine. For molecular weights below 350, biliary elimination is low even with renal obstruction. For molecular weights of 450–850, excretion occurs predominantly in the bile, and little appears in the urine, even if the bile duct is obstructed. Bile also increases absorption from the gut of fatty and lipid-soluble substances. Consuming phenols with lipid often increases their absorption.

IV. EXAMPLES OF COMMON PLANT PHENOLS WITH ACTUAL OR POTENTIAL SIGNIFICANCE IN ANIMAL CONSUMPTION (TOXIC OR BENEFICIAL)

An effort will be made to include here all the common phenolic derivatives of plants having a specific history as toxic substances. Coverage will necessarily be as brief as possible, consistent with presenting a clear overall picture. Physiolog-

ical activity in animals other than toxicity will be noted if it adds to the overall perspective of the phenols as dietary constituents. Groupings are somewhat arbitrary and overlapping.

It has already been indicated that, owing to the evolution of efficient detoxication, the common dietary plant phenols should not be significant as toxicants under normal amounts and conditions, with the possible exception of tannins, which are seen as general deterrents to herbivore feeding. There are, however, limits to the amounts that can be tolerated, and a number of physiological effects have been reported.

A. SMALL PHENOLS, PYROLYSIS PRODUCTS

Phenol itself (Liao and Oehme, 1980), and other small phenols such as catechol, the cresols, guaiacol, orcinols, and pyrogallol, are commonly considered to be industrial chemicals. They may, however, be encountered as natural water constituents (from contact with shale, for example), and they constitute a sizable proportion of the solids of smokes, including smoke from tobacco. They are ingested by breathing smoke, but also by eating smoked foods. Most of the appeal of smoked foods is the flavor, the antioxidant, and the antimicrobial effect of the treatment, which is largely due to the phenols of the smoke.

Pyrolysis of catechin (in gum catechu) and gallates was the historical source of catechol (pyrocatechol) and pyrogallol, respectively. The breakdown of lignin, hydroxycinnamates, and other natural phenols can give small volatile phenols (Singleton and Kratzer, 1973; Tressl *et al.*, 1976). Both microbial breakdown and pyrolysis, for example, give 4-vinylguaiacol by decarboxylation of ferulic acid, and reduction or further reaction can produce the 4-ethyl and 4-methyl analogs and guaiacol. Pyrolysis in air gives vanillin and vanillic acid (Fiddler *et al.*, 1967). Similar products can be produced from other natural cinnamates. Some of these "pyrolysis" products can be produced under relatively mild cooking conditions in foods and beverages. Pyrolysis of nonphenolic materials also produces phenols (Higman *et al.*, 1970). Sodium acetate, protein, or carbohydrate pyrolysis gives phenols, with 60–78% of the product of carbohydrate pyrolysis being phenolic. Not all the products of pyrolysis are simple phenols, of course. Benzpyrene and other arylhydrocarbons are produced, and naphthoquinones, stilbenes, and more complex phenols have been found in smoke tars. Mutagens and comutagens are produced by charring of foods and pyrolysis of food constituents, and the mutagenicity is greater than can be attributed to benzpyrene (Sugimura and Nagao, 1979). However, the active substances do not appear to be phenols, and the pyrolysates of tyrosine and phenylalanine are among the least mutagenic of the amino acids (0.01 of the activity of tryptophan or serine, for example).

Extensive studies in search of the best antiseptics among substituted small

phenols have shown that microbial toxicity is general in phenols, but it varies from weak to strong (Singleton and Kratzer, 1969). Alkyl substituents on the ring tend to reduce animal toxicity and increase the antibacterial effect. Commonly, a second phenolic hydroxyl reduces both the antibacterial effect and animal toxicity. Many microorganisms can oxidize vicinal dihydroxyphenols to open the ring and produce aliphatic dicarboxylic derivatives subject to further metabolism (Dagley, 1967; Ribbons, 1965). This is probably one reason that considerable phenol may be unaccounted for after oral administration to animals.

Phenol content estimated on the surface of smoked meat was about 37 mg/kg, but only about half was recovered, owing to losses in recovery and binding to the meat (Issenberg *et al.*, 1971; Singleton and Kratzer, 1973). Smoke components, probably the phenolic aldehydes such as sinapaldehyde, bind and thus cause considerable loss in dietarily available lysine (Chen and Issenberg, 1972). The toxicity of substituted small phenols in relation to the uncoupling of oxidative phosphorylation, bacterial inhibition, or phytotoxicity can be predicted from physicochemical data on relative acidity and hydrophobicity (Fujita and Nakajima, 1969). The severity of nephrotoxicity of substituted hydroquinones and catechols injected in rats generally increased as their standard oxidation-reduction potential decreased (Calder *et al.*, 1975). Pyrogallol with a low potential was an exception that did not produce renal lesions under the test conditions. Interference with oxidation-reduction systems in the renal cells is the indicated mechanism, but quinone reaction with nucleophiles is an alternative.

Pentachlorophenol, strictly an unnatural phenol, is a potent uncoupler of mitochondrial oxidative phosphorylation, but it also interferes with microsomal detoxication reactions. The latter role appears to be more important, and it can have a synergistic effect with other toxins (Arrhenius *et al.*, 1977). An oral dose of about 157 mg/kg was fatal to man; the oral LD₅₀ for rats is 125–210 mg/kg (Burger, 1966). Phenol, but generally not other monophenols, produced convulsions in experimental animals (Angel and Rogers, 1972). Catechol given intraperitoneally at 80 mg/kg caused tremors and epileptiform convulsions, even in the surgically anesthetized animal. Substituted catechols and certain hydroxynaphthalenes were active but less potent, and pyrogallol was inactive in these investigations (Angel and Rogers, 1972; Angel *et al.*, 1977), but not in earlier studies (Angel and Rogers, 1968). The mechanism may involve catechol-*O*-methyltransferase and interference with the metabolism of catecholamines, but interference with acetylcholine metabolism is also indicated.

The lethal dose for phenol in the adult human is 10–30 gm, and it is about the same for pyrogallol or resorcinol (Moeschlin, 1965). If phenol is spilled over a large surface of the skin, death may occur within 15 minutes. Detoxication of phenol by conjugation is very rapid even when the liver and gut are removed, and up to 600 mg of phenolic material of all types may be ingested per day in the normal human diet (Powell *et al.*, 1974). Feeding rats for 90 days with tenfold

the estimated maximum human dietary content of 1,3-dimethoxybenzene (10 mg/kg per day) and guaiac wood oil (32 mg/kg per day) caused no adverse toxic effects (Oser *et al.*, 1965).

Phenol is nearly equally toxic whether given orally or subcutaneously (LD₅₀ 530 vs 400 for rats) (Singleton and Kratzer, 1969). Parenteral administration is usually ten times as toxic as oral (a rough estimate). Other small phenols are also similarly toxic regardless of the method of administration (e.g., for *o*-cresol the LD₅₀ for rats is 1350 orally, 650 subcutaneously). This is partly because these small phenols readily penetrate skin and other membranes. Phenol is a good solvent for lipids and proteins, and many membranes involve lipoproteins. Topical application to rat skin of phenol, *o*-cresol, *m*-cresol, *p*-cresol, di-cresol, and 2,4-xenol gave LD₅₀ values of 450, 620, 1100, 750, 825, and 1040 mg/kg, respectively (Uzhdavini and Gilev, 1976). Human skin is much less permeable than the skin of experimental animals (Idson, 1973). A balanced lipid-water solubility, a non-ionized state, and modest molecular dimensions are necessary for appreciable penetration of the skin. Penetration of phenol through the skin is speeded up about tenfold once the skin is damaged, and the minimum damaging concentration is much affected by the concentration and the solution in which the phenol is applied (Roberts and Anderson, 1975). The damaging phenol concentration on excised rat skin was 1.4% in liquid paraffin, 7% in water, 6.7% in peanut oil, and 80% in glycerol. Penetration is also shown by induction of liver enzymes after skin exposure only (Kappas and Alvares, 1975).

The risk from small phenols as direct toxicants may not be the only risk involved, particularly in tobacco smoking. Cilia in the lungs sweep mucus and particulate contaminants from the lungs. Ciliary motion seems to be seriously and specifically impaired by smoke phenols (Bernfeld *et al.*, 1964). Furthermore, phenols can serve as cocarcinogens, presumably by helping carry low-solubility carcinogens such as benzpyrene into target cells (Singleton and Kratzer, 1969, 1973). A single application of a carcinogen such as benzpyrene or multiple applications of a phenol solution from tea or from cigarette smoke did not produce significant skin cancer in experimental animals. A single application of benzpyrene, however, followed by repeated applications of phenol, did give a high incidence of cancerous skin tumors (Boutwell, 1967; Kaiser, 1967; Van Duuren *et al.*, 1968). Similarly, increased lung cancer in exposed mice was associated with inclusion of the phenolic fraction in aerosols of tobacco tars (Tye and Stemmer, 1967). Certain phenols accelerate and others inhibit the absorption of benzpyrene into animal skin (Karu *et al.*, 1972). Sugar solutions appear to have nearly equal effects in promoting benzpyrene induction of rat sarcoma, when compared with an aqueous extract of condensed cigarette smoke (Sydnor *et al.*, 1972). Hydroquinone or quinol, a minor metabolite (in conjugated form) of phenol, decreased "takes" and increased the survival of mice receiving melanoma transplants (Chavin *et al.*, 1980).

B. PHENOLIC NUTRIENTS

Tyrosine is considered a semi-indispensable amino acid for animals, since it spares the indispensable phenylalanine and is required in proteins and as a precursor for thyroxine, melanins, and related compounds. In general, an imbalanced excess of an indispensable or semi-indispensable amino acid is less well tolerated in the diet than an imbalance of one of the dispensable amino acids. Tyrosine at 3% or phenylalanine at 4% or more of the diet causes adverse effects in rats (Harper, 1973). Supplementation of a low-protein diet with 5% tyrosine gave rats external pathological lesions in a few days, but substitution of 4-hydroxyphenylpyruvate for the tyrosine gave no signs of toxicity in two weeks (Boctor and Harper, 1968). Low food intake was a symptom and not a cause of tyrosine toxicity, and addition of thyroxine increased the severity of tyrosine toxicity. These data have some value in suggesting maximum tolerance and detoxication levels for an essential constituent phenol.

The water-soluble vitamins have generally been nontoxic, even at abnormally high levels in the diet. Fat-soluble vitamins are not always so benign (DiPalma and Ritchie, 1977; Hayes and Hegsted, 1973). Vitamin E (α -tocopherol, Fig. 2) appears to be relatively safe, however, even at levels higher than are possible with an unsupplemented diet. Consumption controlled to produce double the average plasma level in 28 adults over a period of 3 yr produced no definite bad or good effects. Allergy to vitamin E in an aerosol deodorant is the only known

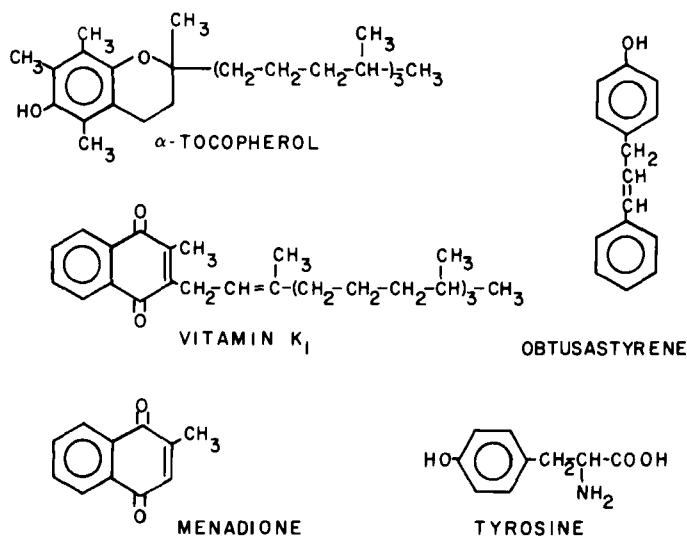


FIG. 2. Structures of vitamin E (α -tocopherol) and vitamin K (K_1 and menadione) active substances, tyrosine (the phenolic amino acid), and an uncommon cinnamylphenol.

toxic reaction to vitamin E in humans. A very high excess in animals can depress growth, thyroid function, mitochondrial respiration rate, bone calcification, and hematocrit and sperm production, while increasing reticulocytosis and causing testicular atrophy. Deficiency symptoms include specific types of liver necrosis, muscular dystrophy, and respiratory deficiency. Adequate vitamin E helps protect against carbon tetrachloride hepatotoxicity, maintain embryonic growth, enhance certain immune responses, and prevent capillary permeability leading to exudative diathesis. The tocopherols, menaquinones, phyloquinone, ubiquinones, and plastoquinones are generally excreted in bile unchanged or in urine as glucuronoside conjugates with the side chain shortened to seven carbons and γ -lactones formed (Wiss and Gloor, 1970).

The natural vitamins K (K_1 , Fig. 2) prevent a hemophilia-like deficiency disease and are involved in respiratory chain electron transport. When injected, K_1 may cause localized nerve paralysis (Boctor and Harper, 1968). Premature infants have been killed by 10 mg of menadiol sodium diphosphate given daily for 3 days (Hayes and Hegsted, 1973). Most of the reports on toxicity in the vitamin K series have been on the synthetic water-soluble menadione (Fig. 2) as a hemolytic oxidant in the body at high doses relative to the dietary K intake. It leads to hemolysis when the natural fat-soluble menaquinones like K_1 do not. It is now known that vitamin K is part of a membrane-bound system that carboxylates glutamate residues in the peptide chains of a number of vitamin K-dependent proteins (Olson and Suttie, 1977; Suttie, 1979). The best known, bovine prothrombin, has 10 of 43 glutamic acid residues carboxylated on the γ -carbon. Dicoumarol interferes with vitamin K in an unknown manner, so that fewer prothrombin glutamates are converted to γ -carboxylglutamate, and the defective prothrombin does not function properly in blood clotting (Suttie, 1979).

Dicoumarol can be considered an antivitamin or vitamin antagonist for vitamin K. Vitamin E antagonists of unknown structure exist in beans. Both vitamins E and K can be antagonized by vitamin A under some circumstances (Somogyi, 1973). In no case are common natural phenols from plants known to be active antagonists of vitamin E or K, and the exact mechanisms of antagonism are not yet clear.

C. HYDROXYCINNAMATES

The common phenolic cinnamates, particularly chlorogenic acid, are not generally present at high levels (coffee beans being an exception at about 3–8%), but they are present in nearly all tissues of higher plants in amounts up to 100 mg/kg fresh weight (Herrmann, 1967). In the course of processing, caffeic acid derivatives (the predominant natural hydroxycinnamate) may be increased; defatted sunflower seed meal has about 2.4% (Mikolajczak *et al.*, 1970). Chlorogenic acid administered orally to cats and rabbits at 1–2 gm/kg or to mice and rats at

4–5 gm/kg caused no symptoms of damage (Chassavent, 1969). The lethal dose given intraperitoneally to mice was 3.5 gm/kg, but this appeared to be due to acidity, because the sodium salt at the same level was well tolerated. The common phenolic cinnamic acids do not ordinarily occur free in plants, but are acylated to quinic acid or other hydroxylic substances. Quinic acid and shikimic acids are among the rare nonbenzenoids that can be converted to benzene derivatives in animals (Brewster *et al.*, 1977). It appears, however, that they are first converted to the saturated cyclohexane carboxylic acid by the intestinal microorganisms of man, mouse, rabbit, guinea pig, or hen (but not ferret—a carnivore) and then metabolized by the liver to the benzoic acid conjugate hippuric acid. The responsible components in the proven carcinogenicity of bracken fern are still uncertain (Wogan and Busby, 1980). Other fractions are definitely active, and the suggestion that part of this activity is due to shikimic acid seems to have been refuted by further studies (Miller and Miller, 1979; Wogan and Busby, 1980).

Chlorogenic acid has been claimed to be allergenic, particularly in coffee; however, it now appears that this was incorrect and that the contamination of chlorogenic acid with coffee protein was the cause (Brewster *et al.*, 1977; Layton *et al.*, 1968). Considering its widespread occurrence, chlorogenic acid must have little allergenic potential, if any. At 1 gm/liter it has been reported to slow digestion by pepsin (Brewster *et al.*, 1977). A mixture of phenolic acids containing caffeic, *p*-coumaric, and ferulic acids (plus vanillic, protocatechuic, *p*-hydroxybenzoic, and *m*-hydroxybenzoic acids) equivalent to the amount contributed by 5% distillers' dried solubles in the diet increased the growth rate of chickens significantly (Dixon and Couch, 1970). In roughages fed to sheep, such as timothy hay and sawdust, vanillic acid, ferulic acid, *p*-coumaric acid, acetovanillone, *p*-hydroxyacetovanillone, and *p*-hydroxybenzaldehyde were present at about 10, 1.5, 1.5, 0.5, 0.5, and 0.005 mg/kg of lignin content, respectively (Fahey *et al.*, 1980). These substances were partly modified or absorbed, since similar values in the feces fell about 10–50%.

Phenolic antioxidants added to foods, notably butylated hydroxyanisole and butylated hydroxytoluene, inhibit the action of some chemical carcinogens when administered before or with the carcinogen. Similar effects have been shown for *p*-coumaric and *o*-coumaric acids (Wattenberg, 1980). The effect is apparently due partly to the enhancement of microsomal-detoxifying enzymes. Caffeic acid and other antioxidant vicinal dihydroxyphenols can also combat toxic reactions by scavenging free radical intermediates. Rao and co-workers have reported (1978) that nitrite in human saliva or rat diet produced nitrosation of added piperazine, oxytetracycline, and aminopyrine. Caffeic acid inhibited these nitrosations by 10–60%.

Ferulic acid had a distinct antiandrogenic activity on the rat prostate, but not on other male accessory sex organs, nor did it have any estrogenic effect on

female rats or mice (Saito *et al.*, 1979). Dihydrocaffeic acid, which is also a metabolite in animals from flavonoids, caffeic acid, and isoferulic acid, but not dihydrocoumaric acid, can substitute for adrenaline as a cofactor in the formation of prostaglandin E from arachidonic acid in an *in vitro* preparation of rat renal medulla (Baumann *et al.*, 1978).

Bile flow, stomach acid secretion, and intestinal motility increase on administration of chlorogenic acid to experimental animals at about 5–100 mg/kg (Brewster *et al.*, 1977). Caffeic acid has generally a similar effect, but quinic acid has not. Injection and oral administration were about equally effective routes for enhancing bile secretion. Cynarin (a dicaffeoyl quinate from artichokes) and the cinnamic acid fraction from wine (caffeoyl) and related tartrates, Singleton *et al.*, 1978) have been recommended as useful stimulants of bile flow, thereby lowering blood cholesterol. This appears to be a property common to many phenolic substances and perhaps to many other substances excreted via the bile, but the lack of toxicity of the common caffeic acid derivatives makes them attractive (Czok and Schulze, 1974; Galecka, 1969; Giraldi *et al.*, 1970; Masquelier and Laparra, 1967). The lipid mobilization associated with fasting was normalized by cynarin and an oat polyphenol preparation (Dorigo and Fassina, 1970; Shulyakovskaya and Stroevasya, 1972). Cynarin has also been claimed to promote urea excretion and favorably affect chemically induced nephritis in the rabbit (Sokolova *et al.*, 1970).

Caffeic acid (but not cinnamic nor ferulic acids) has an antithiamine effect *in vitro*. It seems to be involved in bracken fern toxicity, which can be cured with high thiamine dosage, and in the antithiamine effect of coffee (Somogyi, 1978; Somogyi and Nageli, 1976). However, in other tests caffeic acid did not impair animals with latent thiamine deficiency, and in rats with sufficient thiamine it enhanced the level of thiamine in the body (Schaller *et al.*, 1977; Swanowska and Tautt, 1972). A reported stimulating effect of chlorogenic acid on the central nervous system is minor compared with that of caffeine (Brewster *et al.*, 1977). An effect that increases the volume per pulse, but lowers the pulse rate and blood pressure, appears to be related to the acidity.

Chlorogenic acid is rapidly converted in the animal body to its hydrolysis products caffeic and quinic acids; it was not detected unchanged in the blood stream (Czok *et al.*, 1974). Chlorogenic or caffeic acid gives rise in man to the main metabolites *m*-coumaric acid glucuronide and *m*-hydroxyhippuric acid (Brewster *et al.*, 1977). Animal excretory products include conjugated and unconjugated caffeic, dihydrocaffeic, ferulic, dihydroferulic, vanillic, *m*-coumaric, and 3-hydroxyphenylpropionic acids. The hydrogenation of the α,β double bond and the dehydroxylation reactions may be the result of gut microbe metabolism (Sheline, 1968). Ferulic acid was excreted by the rat following injection both unmodified and as *m*-hydroxyphenylpropionic acid, which involves removal of the 4-hydroxyl, removal of the methyl group from the

3-hydroxyl, and hydrogenation of the side chain (Teuchy and Van Sumere, 1971). Ferulic was not respired to CO_2 and little was incorporated, but it was excreted after intraperitoneal injection—68% in the urine and 19% in the feces. Intravenous administration of *p*-methoxycinnamate to the rabbit gave a half-life of 0.4 hour, and blood level peaked within an hour after oral administration (Woo, 1968). The excretory derivative was *p*-methoxybenzoate. Westendorf and Czok (1978) found that hydroxycinnamates in doses of the order of 5 mg/kg were absorbed from the rat intestine two to ten times as fast as they were eliminated. They were excreted mainly via the urine within 2 hr. The time for 50% elimination was 13–37 min after intravenous administration of about 1 mg/kg. Only about 3% of caffeic and *p*-coumaric acids were excreted in the bile, but excretion of ferulic acid was about 30%. These workers concluded that chlorogenic acid was unlikely to have an important role in enterohepatic circulation.

The antibacterial action of the more usual cinnamates may be significant in some instances (Gupta and Banerjee, 1978; Masquelier and Delaunay, 1965). Unusual cinnamylphenols (obtusastylene, Fig. 2) are potent antimicrobial compounds in nature, and synthetic analogs are being considered for potential uses, including food protection (Jurd *et al.*, 1973; King *et al.*, 1972).

D. GALLIC AND ELLAGIC ACIDS AND RELATED DERIVATIVES

Gallic acid is widely distributed in plants, but the concentration is usually very low. Bound forms of gallic acid, notably epicatechin gallate or galloyl glucoses and tannic acids, are associated with and probably are the main source of free gallic acid in foods. Gallotannic acid is discussed under tannins, and epicatechin gallate is a flavonoid. The acute toxicity of gallic acid is very low; 4–5 gm/kg given subcutaneously or intraperitoneally is required for a 50% lethal dose to mice or rats (Anonymous, 1976; Singleton and Kratzer, 1969). Prolonged consumption in amounts well above normal food levels is not known to produce untoward effects.

After intraperitoneal administration of gallic acid, ethyl gallate, syringic acid, or 3,4,5-trimethoxybenzoic acid (or veratric or vanillic acid) at 150 mg/kg per day for 4 days in rats, no effect on liver microsomal enzyme activity was seen, whereas tannic acid at 25 mg/kg was inhibitory (Gaillard *et al.*, 1974). The major urinary metabolite from gallic acid and gallates including tannic acid is 4-methylgallic acid in rats or rabbits (Booth *et al.*, 1959). A second product is pyrogallol. Syringic acid and 3,4-dimethoxy-5-hydroxybenzoic acids were produced from 3-*O*-methylgallic acid. It is interesting that the animal enzyme appears to favor methylation of the 4-hydroxyl, whereas the plant enzymes methylate the 3 and 5 positions. This may be a methyl-group-sparing mechanism in the animal. Feeding of 1% gallic acid in a diet low in methionine and choline gave rats fatty livers, which were prevented by the addition of choline or methionine

(Booth *et al.*, 1961). A portion of the gallic acid is excreted unchanged, but the glucuronoside is also formed, and the total excretion of glucuronic acid is increased tenfold upon oral intake of gallic acid by rabbits (Blumenburg and Dohrmann, 1960).

Gallates potentiate adrenaline toxicity (Baraboi, 1967), a property shared with several other readily soluble phenols, presumably by interfering with adrenaline's detoxication by methylation, since catechol *O*-methyltransferase is inhibited (Dorris and Dill, 1977). Gallic acid complexes with iron to make blue-black inks, but in the diet gallic acid can increase the regeneration of hemoglobin in anemic rats, presumably by making the iron more available and reducing it to the ferrous form (Stoewsand and Hrazdina, 1972). Gallic acid (4%) strongly inhibited acid secretion from the perfused stomach of rats as stimulated by intravenous tetragastrin (Tani, 1978). Tannic acid behaved similarly, but required higher concentration (8%). Protocatechuic, *p*-hydroxybenzoic, and salicylic acid or aspirin stimulated the response to tetragastrin and sometimes enhanced the basal acid secretion.

Gallic acid derivatives, particularly esters, have appreciable activity against bacteria and fungi (Carlson *et al.*, 1951). Along with related esters of *p*-hydroxybenzoic acid, they have been used to stabilize cosmetics and foods, but they can produce crystalline deposits owing to their low solubility (Hinneken and Vermeulen, 1966-1967). Contact dermatitis of an allergic type is produced in certain individuals by the latter substances (Goldberg, 1970).

Bradykinin, a vasoactive peptide which induces contractions in smooth muscle, is antagonized by gallic acid and its esters as well as by certain tranquilizers (Posati *et al.*, 1970). Gallates have been reported to have about 3% of the anti-inflammatory activity of hydrocortisone; they have also been used to inhibit spasms, to treat swellings, and to protect against radiation damage (Chernetskii *et al.*, 1967; Saeed *et al.*, 1974). Gallic acid suppressed the *in vitro* immune response to antigens through its effect on the thymus lymphocytes (Archer *et al.*, 1977).

Protocatechuic (3,4-dihydroxybenzoic) acid is metabolized in man to vanillic and isovanillic (3-methyl-4-hydroxy- and 3-hydroxy-4-methoxybenzoic) acid conjugates in the ratio of about 12.8 : 1 after oral consumption of 1-gm doses (Price, 1969). If isovanillic acid is eaten, some 3,4-dimethoxybenzoic acid is produced. Vanillin orally administered to rats was recovered 47% as vanillic acid, 19% as vanillyl alcohol, 10% as vanilloylglycine, 8% as catechol, 7% unchanged, 2% as 4-methylcatechol, 0.6% as 4-methylquaiacol, and 0.5% as guaiacol, for a total recovery of 94% (Strand and Scheline, 1975). These metabolites occurred mostly as glucuronide and sulfate conjugates, and the glucuronide conjugates that were excreted in the bile were metabolized by intestinal bacteria to the toluene derivatives and decarboxylated products. Ethyl vanillate is an antimicrobial agent active against fungi and food spoilage organisms. Its LD₅₀

for rabbits is about 1.6 gm/kg, for guinea pigs about 2.2 gm/kg, and the maximum tolerated by rats is about 3 gm/kg per day for an 18-day period (Christie *et al.*, 1951). The effects of toxic doses include generalized engorgement of the capillary bed and hemorrhage followed by necrosis of the mucosa in the upper gastrointestinal tract. Myocarditis, renal degeneration, and thickening of pulmonary alveoli are seen also. Ethyl vanillate has been used against progressive infection of humans by *Histoplasma capsulatum* and has been credited with saving the lives of 5 out of 12 terminally ill patients (Christie *et al.*, 1951). The curative dose was only 25–30% below the toxic dose.

Ellagic acid is very poorly soluble in physiologically tolerated solutions, and oral toxicity appears to be nil under practical conditions. Very low levels of ellagic acid (10^{-4} M) *in vitro* or injected intravenously produce a hypercoagulable state in blood by activating the Hageman factor XII of the clotting system (Botti *et al.*, 1965; Girolami *et al.*, 1977). This ordinarily does not lead to platelet aggregation, thrombosis, or embolism in the animal, but it can minimize traumatic bleeding, and it speeds clotting. The activated clotting effect decays rapidly, but injection of ellagic acid on the ninth or thirteenth day after whole-body irradiation decreased the intensity of hemorrhage, compared with that in control animals without ellagic acid (Pospisil *et al.*, 1969).

Ellagic acid at 1.2 mg/kg given intravenously to mice on the eighth or sixteenth day of pregnancy increased the incidence of abortion (Moe, 1971). Suppression by ellagic acid of anaphylaxis, hypersensitive immune reactions, and inflammation edema induced by carrageenin have been reported (Briseid *et al.*, 1971; Schwartz and Zimmerman, 1971). On the other hand, injection of ellagic acid directly into joints produces inflammation, which is countered by aspirin (Van Arman *et al.*, 1970). The effects of ellagic acid on experimental carcinomas have been variously promoting or inhibiting; they seem to be related to effects on coagulation and blood supply. However, the hypercoagulation effect requires the lactone bonds and is destroyed by acetylation of the four phenolic hydroxyls (Botti *et al.*, 1965). Taspine, an alkaloid with a methoxylated ellagic skeleton, is an anti-inflammatory agent and inhibits the reverse transcriptase, RNA-directed DNA polymerase, of several animal tumor viruses (Sethi, 1977).

Ellagic acid administered orally to normal rats did not appear in the feces or urine, and only a small amount was recovered from the feces of germ-free rats (Doyle and Griffiths, 1980). About 10% of the dose was excreted in the urine as the product with two fewer hydroxyls and one fewer lactone function, 3,8-dihydroxy-6*H*-dibenz(*b,d*)pyran-6-one. A second metabolite was found but not identified; traces of sulfate conjugation products were also observed. Injection gave an additional metabolite in the urine. Both metabolites produced by oral administration were not found in germ-free animals, but they were produced when ellagic acid was incubated with normal feces, indicating that they are products of microbial action.

E. FLAVONOIDS

1. General Nutritional Aspects and Toxicology

The qualitative flavonoid composition of foods, some data on the amounts present, and the roles of flavonoids in foods have been reviewed periodically (Bate-Smith, 1954, 1959; Harborne, 1979; Heintze, 1965; Herrmann, 1959, 1976; Kühnau, 1976; Swain, 1962). Common plant foods contain from traces to several grams of flavonoids per kilogram fresh weight. A few hundred milligrams per kilogram is common for many vegetables and fruits. The human dietary intake has been estimated at about 1 gm/day, with nearly half of this as total biflavans (which we consider condensed tannins) and the rest about equally divided (200 mg/day each) among anthocyanins, catechins, and 4-oxoflavonoids (flavonols, flavanones, and flavones) (Kühnau, 1976). Animals consuming leafy plants may have much higher proportional intakes. Buckwheat may be as high as 5% rutin, leaves of rue as high as 2.2% rutin dry weight, and Chinese lettuce leaves 5.6 gm/kg, fresh, in flavonoids. Individual seedling plants and plant parts may have very different qualitative patterns and relative contents of the different types of flavonoids. Clonally propagated varieties often can be identified by their flavonoid pattern—chemotaxonomy carried to the ultimate.

The flavonoids contribute color, flavor, and processing characteristics important in food. They are ordinarily of extremely low toxicity. Broiler chickens fed diets with 2.5% citrus flavonoids were unaffected; 5.0% caused a reduction in growth (Deyoe *et al.*, 1962). Anthocyanin preparations from berries at 20 gm/kg given orally to rats and mice caused no deaths. No abnormalities were produced in rats fed 6 gm/kg daily for 3 mo (Pourrat *et al.*, 1967). The LD₅₀ values in mice and rats were 4.11 and 2.35 gm/kg for intraperitoneal injection and 840 and 240 mg/kg for intravenous, respectively. No teratogenic effect was found over three generations of rats, mice, and rabbits. Enhanced capillary resistance was greater than it was with rutin, and intravenous injections at 200 mg/kg caused vasodilation 2% that of adenosine, transient reduction of blood pressure, a decrease in respiratory amplitude, and diuresis.

Quercetin, rutin, hesperidin, and especially tricetin stimulated the basal metabolic rate when fed to rats (Stelig and Qasim, 1973). Naturally occurring in *Prunus japonica* fruits, acetylated kaempferol 3-rhamnoglucoside was a strong purgative, but it lost most of its activity when the acetyl group was removed (Haruji *et al.*, 1975). An earlier finding of cataract formation after administration of rutin was shown to be due to impurities in the preparation (Nakagawa *et al.*, 1965); in fact, dietary flavonoids appear to protect against diabetogenic cataract. Quercitrin fed to *Octodon degus* rodents at 2.5 gm/kg of diet inhibited aldose reductase and the buildup of lens polyols, which causes

cataracts (Varma *et al.*, 1977). Similarly, gossypetin-8-glucoside from hibiscus flowers given at 1.5% in a 30% galactose diet for rats inhibited the enzyme, decreased galactitol deposition, and delayed, but did not prevent, cataract formation (Parmar and Ghosh, 1979). Absorption or excretion of galactose, itself, was not affected. Chemically modified to increase solubility, derivatives of quercitrin had effects on cataract prevention similar to those of quercitrin and greater than those found with rutin analogs (Fauran *et al.*, 1978; Varma *et al.*, 1978). Some promise for preventive therapy or nutrition appears to be offered.

Perinatal fatalities in human infants have been caused by supradietary parenteral dosage of the pregnant mother with rutin. The fatalities appear to have been caused by the insolubility of rutin and its formation of concretions within the liver and gall bladder, and by nephrotoxicity (Pfeifer *et al.*, 1969, 1970). The more soluble derivative, rutin sulfate, did not give concretions, but caused other toxic effects, while the soluble semisynthetic derivative of rutin with three (7,3',4') phenolic hydroxyls converted to β -hydroxyethoxy groups was well tolerated, even in high doses. A mixture of the latter substance at 150 mg/kg per day plus coumarin at 25 mg/kg per day, injected from the sixth to the thirtieth day of gestation in pigs, produced no effects attributable to the medication in the mother or the offspring (Grote *et al.*, 1977).

Other selected examples of low toxicity or of mild negative effects might be cited, as well as more observations on the positive effects of flavonoids, but it has been generally agreed that the common flavonoids have very low toxicity when given orally, and it is usually still quite low when they are given parenterally (Bauer, 1978). In fact, the low order of toxicity and the mild but diverse and reportedly favorable physiological and therapeutic effects of flavonoids have promoted considerable search for synthetic or partially synthetic analogs with useful drug action.

2. Bioflavonoids and Flavonoids as Drugs

The history of flavonoids as dietary constituents has been marred by considerable and continuing controversy regarding their role in relation to vitamin C, scurvy, and capillary bleeding. In 1936 Szent-Györgyi and co-workers reported (Rusznayk and Szent-Györgyi, 1936) that flavonoid preparations from paprika and citrus peel ("citrin") could restore complete health to scorbutic guinea pigs when vitamin C alone did not. Particularly, a tendency of the capillaries to ooze blood after minor injury was corrected by the flavonoids along with vitamin C, and not by ascorbic acid alone. The flavonoids were termed vitamin P (for permeability). These reports were soon followed by both contrary and corroborative findings and by many reports on different flavonoids and other applications. By 1959, over 1000 papers had appeared; a number of reviews interpret this history (Anonymous, 1969; Böhm, 1959, 1960; Hughes and Wilson,

1977; Kefford, 1973; Kefford and Chandler, 1970; Orzechowski, 1962; Vogin, 1960).

In 1950, the American Society of Biological Chemists and the American Institute of Nutrition proscribed the term vitamin P on the basis that true vitamin activity was not exhibited. The term is still used, particularly in Russian literature, but it has largely been replaced by "bioflavonoid." Further confusion arises from the fact that some authors claim that other phenolic substances, such as certain coumarins (notably esculetin), have "bioflavonoid" effects. Other workers consider planar benzpyrones to be the active structure, but they include catechins. Poor interaction among chemists, biologists, statisticians, and clinicians has plagued this subject more than most. In 1968, the United States Food and Drug Administration withdrew approval for use of bioflavonoids as a drug, even though no serious side effects were known, because it was considered that there was no proven efficacy in man for any clinical purpose. Since then, clinical use and experimentation have been minimal in the United States, but they continue apace in other countries, particularly in France, Germany, Hungary, and Russia (Comèl and Laszt, 1972; Farkas *et al.*, 1975, 1977).

It seems clear that only vitamin C is curative for acute scurvy, but it is also true that flavonoids can have a favorable effect on chronic, borderline vitamin C deficiency. This vitamin C-sparing activity was known before 1936, and the active flavonoids were named vitamin C₂, a term still used in France (Farkas *et al.*, 1975, 1977; Kühnau, 1976; Masquelier *et al.*, 1973). Flavonoids of all common classes have been reported to have bioflavonoid activity, including, in increasing order, flavanones, anthocyanins, flavonols, leucoanthocyanidins, and catechins. Quercetin and its derivatives and catechins have been most studied, since they are more cheaply available and are quite active.

When bioflavonoids at 50 mg/kg per day are administered to guinea pigs with a suboptimal level (1 mg/day) of ascorbic acid in the diet, and the results are compared with those for the same diet devoid of flavonoids, the animals receiving the flavonoids have a higher ascorbic acid content, particularly in the adrenals, spleen, liver, kidney, and leukocytes (Wilson *et al.*, 1976; Zloch, 1974). Increased accumulation and retention in the reduced form in guinea pigs were also found for the unnatural D-isoascorbic acid administered orally with rutin and epicatechin (Zloch and Ginter, 1979). The bioflavonoids have other effects, but they may spare ascorbic acid by helping to keep dehydroascorbic reduced to ascorbic acid (Hughes and Wilson, 1977). The ratio between the two acids in animal tissues does seem relatively reduced under the influence of bioflavonoids. Flavonoids also can have an antioxidant effect in the diet itself (Bate-Smith, 1954; Kühnau, 1976) and can help conserve minimal levels of ascorbic acid. Quercetin and many other active flavonoids are good chelators of copper and other heavy metal ions that catalyze rapid destruction of ascorbic acid (Bate-Smith, 1954; Hughes and Wilson, 1977; Kühnau, 1976). Cavallini *et al.* (1978)

found that quercetin and taxifolin were remarkably active, quercitrin less so, in protecting rat liver microsomal and mitochondrial preparations from peroxidizing systems by free radical scavenging and not by metal chelation.

The effects of flavonoids in animals have probably been too closely and unnecessarily associated with vitamin C (Hughes and Wilson, 1977). There are many other physiological effects of flavonoids in animals receiving an adequate supply of ascorbic acid. Willaman listed 33 types of biological and biochemical activities reported for flavonoids in 1955, and Böhm listed 40 by 1960. Although a few of these are either very limited in occurrence or have not stood the test of time, others are continually being added to the list or examined in more detail (Bauer, 1978). Havsteen (1980) has reviewed some specific biochemical effects of flavonoids in relation to medical applications.

The most studied effects of flavonoids are related to the vascular system, particularly the decrease in pathological capillary fragility or the increase in resistance of normal capillaries to trauma (Comèl and Laszt, 1972; Fairbairn, 1959; Farkas *et al.*, 1975, 1977; Gabor, 1974; Gazave *et al.*, 1974; Martin and Szent-Györgyi, 1955). Response to a small local skin injury or irritation involves capillary dilation (reddening) and edema (swelling, leakage of plasma proteins into surrounding tissue). Bioflavonoids resist these changes, apparently by more than one mechanism. They tend to maintain the normal tensile strength of capillary walls. They may antagonize histamine's effect in inducing capillary permeability (but they are not specific antihistamine drugs). They appear to close capillary sphincters and to restrict blood entry by prolonging catecholamine action. They may affect collagen formation. They appear to affect membrane integrity, permeability and some forms of active transport across cellular membranes. Exhaustive review of these areas is not possible, owing to voluminous and somewhat conflicting reports, but in order to relate physiological effect to potential toxicity, some discussion seems required.

Catechin, reported as one of the most potent bioflavonoids, occurs as the free aglycone and is fairly water-soluble, but it is also relatively lipid-soluble and apparently can adsorb to cells, interact with membrane proteins and lipids, and affect permeability in both directions across the membrane (Farkas *et al.*, 1977; Ring *et al.*, 1976).

Collagen is the major organic constituent of the blood vessel wall, and catechin stabilizes it in terms of shrinkage temperature, etc., apparently by an adsorptive tanning and cross-linking process (Comèl and Laszt, 1972). The fact that biflavans (small condensed tannins) are quite active in capillary strengthening, especially if injected, also suggests phenol-protein binding as part of the effect on collagen. Indirect effects may include sparing of vitamin C, since ascorbic acid has a clearly established role in the collagen (hydroxyproline)-forming lysine-proline hydroxylation system (Farkas *et al.*, 1977; Hughes and Wilson, 1977; Kühnau, 1976). Flavonoids in the diet have a normalizing, protec-

tive role against abnormal collagen formation in lathyrism, a poisoning with natural β -aminopropionitrile leading to aortal aneurism (Cetta *et al.*, 1972; Gendre *et al.*, 1977). Catechin appears to have a general protective effect on collagen and the connective tissue system (Farkas *et al.*, 1977). This effect appears to be related to adsorption affinity of the protein-tannin type, but it also involves hydroxyproline synthesis.

Blumenkrantz and Asboe-Hansen (1978) found that catechin in the medium was inhibitory to collagen biosynthesis in chick tibia cultures. Decreased collagen formation was due to decreased biosynthesis of the procollagen, decreased activity of the hydroxylating enzymes, and chelation of ferrous ions. Catechin was recommended for testing in diseases involving increased collagen synthesis, such as scleroderma.

Effects that may be related to normalizing collagen include findings by Hladovec (1977) that exfoliation of endothelial cells into blood was inhibited by parenterally administered flavonoids in rats. This exfoliation is enhanced in humans by cigarette smoking, and oral administration of hydroxyethylrutosides suppressed the effect (Prerovsky and Hladovec, 1979). Daily oral administration of a mixture of 30 mg of coumarin and 180 mg of a hydroxyethyl derivative of rutin for periods of 1-54 mo produced significant softening of the arm tissue in patients after mastectomy (Clodius and Piller, 1979). This mixture and similar preparations involving hydroxyethylrutins have been widely used for some time experimentally and clinically, and in general medicine in Europe, with few undesirable effects noted (Scholten, 1979; Voelter and Jung, 1978).

In experimental acute pancreatitis in dogs, only those that received injections of tris-*O*-hydroxyethylrutins plus coumarin survived, and both pancreatic edema and necrosis were reduced by the treatment (Bartos *et al.*, 1979). Robinin and hyperin injections lengthened the average survival time of mice subjected to bilateral nephrectomy (Sokolova *et al.*, 1978). Rats on a flavonoid-free diet showed prolonged effects from caffeine, harmine, morphine, hexobarbital, and pentobarbital, compared with those on a normal diet (Seidel and Endell, 1978). The results were attributed to activation of liver metabolism by the flavonoids. The rats on the flavonoid-free diet over 25 wk grew significantly more in body weight and lipid content than did those on the normal diet, but no data were given on the effects of subsequent addition of flavonoid to the animals on the flavonoid-free diet. Silybin, an unusual coniferyl alcohol derivative of dihydroquercetin, if administered very soon after *Amanita* mushroom poison in rats, mice, and dogs, produces an antitoxic effect apparently by inhibiting uptake of the toxin by the liver and increasing its urinary excretion (Faulstich *et al.*, 1980).

Potentialization of catecholamines by interference with their inactivation is one possible mechanism for the reduction of capillary permeability and the spasmolytic activities of flavonoids. This interference appears to occur in more than one manner. As was already noted, phenols in general are often substrates for detoxi-

cation by methylation. Methylation occurs during the digestion and metabolism of flavonoids and would compete with catecholamines for a limited methyl group pool (Gazave and Parrot, 1973a,b). However, some flavonoids appear to be inhibitors of catechol *O*-methyltransferase, and thus prolongers of the catecholamine effects, without serving as substrates (Kühnau, 1976). A catechol B-ring and aglycone rather than glycoside were more inhibitory, but some activity was shown by all chalcones and flavonoids (Gugler and Dengler, 1973).

Feeding of epicatechin to rats on a choline-deficient diet prevented a fall in choline level in blood and liver, normalized the lipid content of the liver, and decreased the cholesterol content (Chaudhari and Hatwalne, 1977). This apparently opposite effect might result from diminished methyl group consumption through *O*-methyltransferase inhibition, but serious nervous effects were not reported. Inhibition of oxidation of catecholamine derivatives by flavonoids may also be involved, but interference with the uptake of catecholamines into adrenergic nerve terminals appears not to be affected by flavonoids (Jayasundar *et al.*, 1977).

The effects of flavonoids on blood vessels and edema combine to give an anti-inflammatory action (Gabor, 1972, 1974). Reports of the beneficial effects of flavonoids in many experimental conditions have often been followed by clinical application. Conditions include prevention of or aid in recovery from frostbite, myocardial infarction, thrombophlebitis, scalding, "little strokes," bruising in contact sports, and irradiation damage. Bioflavonoids given orally in the final stages of Rh-factor-threatened pregnancies have been reported to reduce antibody formation by decreasing the contact between fetal and maternal blood. Venous insufficiency and varicosity problems have been treated rather widely in Europe with sizable and prolonged dosage with flavonoids. Only with parenteral administration, and then apparently only when solubility problems are involved, have any serious problems of toxicity appeared.

Injected flavonoids have also been reported to produce a transient lowering of arterial blood pressure, a slowing of the heart rate, and yet an increase in the amplitude of the pulses and blood flow. Flavonoids inhibit erythrocyte aggregation (Robbins, 1977) thereby lowering the blood viscosity slightly (Farkas *et al.*, 1977). Methoxylated flavonoids such as nobiletin and tangeretin (Fig. 3) of citrus fruit appear to be the most active (Robbins *et al.*, 1971), and flavonoids increase survival of rats on thrombogenic diets (Robbins, 1967). Flavonoids and certain other phenols antagonize the spasmogenic activity of prostaglandins or affect the conversion of arachidonic acid to prostaglandins; this is probably also related to their anti-inflammatory activity (Damas *et al.*, 1977; Levine and Hong, 1977; Lindgren *et al.*, 1977; Murrari *et al.*, 1972). Baumann *et al.* (1979) found many phenols, including catechin and the cresols, to be inhibitory, but others, including rutin, dihydrocaffeic acid, and *p*-hydroxybenzoic acid, to be stimulatory to rat renal medulla prostaglandin synthetase. Dihydrocaffeic acid, which can be a

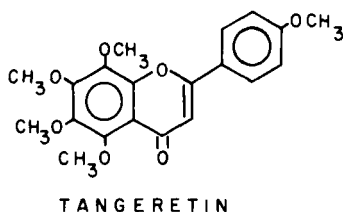


FIG. 3. The structure of tangeretin, an example of relatively rare, fully methoxylated flavones of nutritional significance only in the peel of various citrus fruits. (Nobiletin = 3'-methoxytangeretin.)

metabolite of flavonoids in animals, had about the same activity as adrenaline. The dimethoxy derivative was without stimulatory effect.

Flavonoid-3-glucosides, but not aglycones, appear to release endogenous histamine when injected (Lecomte, 1971). Quercetin given intraperitoneally at 25–100 mg/kg protected guinea pigs against bronchospasm from coadministered histamine (Marozzi *et al.*, 1970), but generally flavonoids have not appreciably modified anaphylaxis. Catechin at concentrations of 10^{-3} M or less markedly inhibits histidine decarboxylase, the producer of histamine, without inhibiting histamine-inactivating enzymes (Lorenz *et al.*, 1973). The incidence of experimental stress ulcers in rats was reduced about 80% by catechin administered intraperitoneally at 1–50 mg/kg, which blocked histamine formation (Reimann *et al.*, 1977). Unusual isoprenylflavonoids had specific inhibitory effects on experimental gastric ulceration in rats (Sasajima *et al.*, 1978). Histamine appears to be a control mechanism for gastric secretion, and clinical trial to suppress gastric hypersecretion was recommended, since catechin is so low in toxicity. Flavonol aglycones including quercetin strongly inhibit induction of histamine secretion from rat mast cells and appear to act by inhibiting calcium-ion active transport ATPase (Fewtrell and Gomperts, 1977a,b). Calcium entry appears to trigger the histamine release.

The effect of flavonols with membrane active transport ATPases may be general; for example, the sodium-potassium ATPase of rat brain microsomes is inhibited (Mirsalikhova and Pakudina, 1977). Wuethrich and Schatzmann (1980) found that active transport across human erythrocyte membranes of Ca^{2+} , Mg^{2+} , and $\text{Na}^{+}\text{-K}^{+}$ was inhibited by quercetin. ATPases with different characteristics were involved. Spector and co-workers (1980) purified the $\text{Na}^{+}\text{-K}^{+}$ ATPase from Ehrlich ascites tumor cells and incorporated it into liposomes, which then weakly catalyzed the ATP-dependent influx of Na^{+} . Addition of quercetin at 24 $\mu\text{g}/\text{mg}$ of protein plus lipid gave a severalfold increase in the efficiency of pumping Na^{+} per ATP by the preparation, but had no effect on similar but already efficient ATPase preparations from electric eel or mouse brain. Quercetin may be acting as a regulatory subunit that protects the membrane enzyme against the

entry of water. The relative inefficiency of the tumor ATPase appears to be related to the high aerobic glycolysis of tumors. Flavones, in general, at relatively high concentrations (800–1000 nmol/mg of protein) inhibited mitochondrial ATPase (Bohmont and Pardini, 1979).

Flavonoids affect carbohydrate and fat metabolism and ATP production in several ways, and generally it is more than a "simple" decoupling of oxidative phosphorylation (Farkas *et al.*, 1977; Gajdos *et al.*, 1969). Tumor cells have an abnormally high aerobic glycolysis, which is regularized by quercetin, which also inhibits tumor cell proliferation at concentrations of 5–20 mg/liter (Suolinna *et al.*, 1974, 1975). Bioflavonoids such as quercetin inhibit mitochondrial hexokinase activity without changing the ATPase activity (Graziani, 1977). Quercetin occurs free and bound in complex with calcium and other metal ions. The enzymic influence of quercetin depends on the metal ion concentration; it appears that the metal complex inhibits whereas the free flavonol stimulates ATP hydrolysis (Mal'yan and Akula, 1977).

Flavonoids can have a rather specific action on animal metabolism and membrane transport, frequently involving the carbohydrates. Phlorizin, a botanically unusual dihydrochalcone glucoside, has a specific "diabetes-like" effect on glucose excretion by animals, as is well known (Singleton and Kratzer, 1969, 1973). The rapid excretion by Ehrlich ascites tumor cells of lactic acid via a lactate-proton mechanism is potently inhibited by quercetin and related compounds (Belt *et al.*, 1979). Flavonoids with four or five hydroxyl groups and an intact C-2–C-3 double bond, and without glycosidation, were most active. At about 0.1 $\mu\text{g}/\text{mg}$ of protein, quercetin inhibited lactate efflux by 50%. Apparently, a lowered internal cell pH is involved. The inhibition of aldose reductase and the delay of cataracts have already been discussed. Naringenin appears to have direct effects on the membranes as well as general metabolic effects in dog renal cortex slices (Robinson *et al.*, 1979), as measured by oxygen consumption and accumulation of glycine and glucoside, hippuric acid, and nicotinamide derivatives.

Cyclic adenosine monophosphate (cAMP) affects many aspects of animal metabolism, notably the mobilization of glucose 1-phosphate from glycogen. Among 17 flavonoids, quercetin and kaempferol were the most active in inhibiting cAMP phosphodiesterase, the enzyme that hydrolyzes cAMP to AMP thus deactivating it (Beretz *et al.*, 1978). Their activity approached that of papaverine, and several other flavonoids were active in the micromolar range. Additional studies added cyanidin chloride to the very active list and showed that the effects of the active flavonoids were competitive (Ferrell *et al.*, 1979). The related guanylic nucleotide, cGMP, phosphodiesterase was even more inhibited than the cAMP enzyme, with (+)-catechin especially selective between the two. Amentoflavone potency was 100 times as high as that of the other flavonoids, and five to ten times as inhibitory as papaverine in separate tests with

cAMP (Ruckstuhl *et al.*, 1979). It is believed that the active flavonoids compete with cAMP for a nucleic acid binding site at which stacking occurs (Ferrell *et al.*, 1979). Activity among the tested compounds was flavonols > flavones or anthocyanidins > flavanones or flavanonols > catechin (Beretz *et al.*, 1978). The dietary significance, if any, of these findings is not yet clear, but cAMP affects many aspects of animal metabolism, and the activity is high. Catecholamines like adrenaline activate adenylate cyclase, resulting in increased cAMP, a 1- μ mol increase of which, per kilogram of muscle, causes the formation of 25,000 times as much glucose 1-phosphate per unit time. A high level of cAMP is also associated with a decreased nerve response to excitatory stimuli. Inhibition of hydrolysis of cAMP would intensify or prolong the reactions.

3. Mutagenicity

Older reports of treatment of experimental cancer are variable, some reporting inhibition of "take" or growth of transplanted cancers by certain flavonoids, others reporting stimulation or no effect. This situation is common to other phenols, especially coumarins. For example, DiGiovanni *et al.* (1978) reported that quercetin inhibited by 22%, whereas myricetin, by 54%, and 4',5,7-trihydroxyflavone, by 29%, enhanced skin tumor initiation when applied topically to shaved mice before application of a benzantracene carcinogen. There has been sufficient indication of antineoplastic and cytotoxic effects, particularly among uncommon flavones (Kühnau, 1976), to initiate continuing searches among nonfood plants for useful, unusual flavonoids (Dobberstein *et al.*, 1977) and for the preparation of synthetic analogs (Slaga *et al.*, 1977), but the common flavonoids do not appear to be useful anticancer drugs (Fritz-Niggli and Rao, 1977). The variable results may be due in part to the fact that low levels of flavonoids can stimulate cell proliferation, whereas higher levels, particularly if quinones are formed, inhibit normal cell multiplication, according to *in vitro* studies (Huot *et al.*, 1974). Also, only aglycones appear to be potentially active, and glycosides occur more commonly (Kühnau, 1976).

That common flavonoids apparently have little useful or specific effect against neoplasms is some comfort, because many antitumor drugs are carcinogens (Newberne *et al.*, 1978). The tumor-promoting or cocarcinogenic effect of tea flavonoid derivatives has already been mentioned and has been tentatively attributed to aid in penetration of the active carcinogen to the sensitive site. Somewhat surprisingly, there is evidence that some tris-*O*-(β -hydroxyethyl)rut-oxide can penetrate from gel or ointment through human skin (Pratzel, 1972; Wasilewski, 1972).

Recently, different groups of workers have reported on the mutagenic activity of flavonoids on *Salmonella typhimurium* mutants—the Ames test. In one study, naringin, rutin, neohesperidin, hesperetin, dihydroquercetin, and permethyl

quercetin were inactive with or without liver microsomes. Only quercetin, of those tested, caused a reversion of mutants (that is, mutation) without metabolic activation by liver microsomes (Bjeldanes and Chang, 1977). Microsomal activation increased the mutagenicity of quercetin threefold and converted quercetin pentaacetate to a mutagen. Activated quercetin was 0.1–0.001 as active as the recognized potent carcinogens 2-aminofluorene and aflatoxin. Two hydroxyphenylacetic acids, metabolites of quercetin, were inactive.

Another group reported kaempferol, quercetin, and galangin to be mutagenic, but removal of a hydroxyl from galangin (3,5,7-trihydroxyflavone) greatly reduced or destroyed the mutagenic activity (Sugimura *et al.*, 1977). A third group found frameshift mutation from the flavonols quercetin > myricetin > rhamnetin > kaempferol > fisetin > morin > robinetin > apigenin (Brown *et al.*, 1977). Taxifolin, dihydroquercetin, fell between fisetin and morin, and diosmetin and galangin were inactive. All the mutagenic flavonoids were significantly activated by microsomes. Glycosides were weakly active, but they increased 10- to 20-fold in mutagenicity when incubated with gut bacterial enzymes (presumably glycosidases included) and microsomes. The bacterial mutagen of a Japanese spice from seeds of sumac has been shown to be quercetin (Seino *et al.*, 1978), and that from a Japanese vegetable pickle was kaempferol and isorhametin (Takahashi *et al.*, 1979). The Japanese pickle mixture was, however, only about one-sixth as mutagenic as a Chinese pickled product it resembled, and the Chinese sample was heavily contaminated with a fungus known to produce nitrosamines.

Hardigree and Epler (1978) also found quercetin and myricetin to be active without liver microsomal activation, although more so after, whereas kaempferol, rutin, morin, and fisetin required microsomal activation to show reversion mutation rates above the control rate. Induced reversions with these compounds occurred only with the frameshift mutant bacteria and not with missense mutants. Macgregor and Jurd (1978) tested forty compounds related to quercetin in the *Salmonella typhimurium* strain TA98 assay and found four (quercetin, myricetin, rhamnetin, and 5,7-di-*O*-methyl-quercetin) that were unequivocally mutagenic without metabolic activation, and six that were mutagenic with activation (galangin, kaempferol, tamarixetin, morin, 3'-*O*-methylquercetin, and 4',7-di-*O*-methylquercetin). They concluded that mutagenicity required a flavonol structure (flavonoid with 4-keto, free 3-OH, 2-3 double bond) and a 5-OH group or other configuration that weakened the hydrogen bonding between the 3-OH and the 4-keto groups, allowing the 3-OH to tautomerize to the 3-keto form. Although free B-ring hydroxyl groups were not essential if a rat liver metabolic activating system was employed, the active form appears to require a B-ring that permits oxidation to a quinoid form. They suggest that quinone methide forms or hydroperoxides may be the proximate mutagen, and note that it is evidently not quercetin itself, owing to considerable

increased mutagenicity after activation by rat liver microsome preparations. So far, it appears that flavonoids in general are not mutagens in this bacterial test; even flavonols are often weak or inactive mutagens requiring activation with primed microsomes or bacterial enzymes, and activation requires removal of the glycoside groups normally attached to flavonols in the plant.

The significance of these results remains to be seen. It is difficult to believe that, as ubiquitous as quercetin is in the normal diet of all plant-eating creatures, it can be a significant carcinogen. Few indications of such an effect have been forthcoming from animal testing, as far as this reviewer knows. There is a report that bladder implants of solid quercetin might give cancer in mice (Boyland *et al.*, 1964). Quercetin and other flavonoids tested did not increase the normal frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice given up to 1 gm/kg by mouth (Macgregor, 1979). The same result was obtained by quercetin injected intraperitoneally. Rat urine after a similar dosage was mutagenic to the *Salmonella* strains TA98A and TA100, indicating the presence of the mutagen in the animal. Brown (1980) and Sugimura and Nagao (1979) have also reviewed the situation. A study in progress indicates no increases in numbers of tumors in mice on a 2% quercetin diet. More rare types of tumors may be present in the test group (Brown, 1980).

It is usually accepted as axiomatic that essentially all mutagenic substances have been or will be shown to be carcinogenic. Certainly, concern and careful investigation are mandated by indications of mutagenicity. Activation by bacterial preparations can involve removal of β -glycoside groups that animals alone cannot hydrolyze. Further extensive metabolic breakdown and/or conjugation, however, would generally follow in the normal dietary situation. The rat liver microsomal preparations used for activating quercetin in these tests were taken from rats injected with polychlorinated biphenyl, β -naphthoflavone, or phenobarbital a few days previously (Sugimura and Nagao, 1979). Would the results be the same if quercetin were used to condition the livers to be utilized as activator for the bacterial mutagens? The bacteria are themselves already mutants produced by introduction of a plasmid (Sugimura and Nagao, 1979) and are nutritionally deficient, so that reversion will lead to growth and recognition. Mutagenesis in this system could be viewed as a repair process. It is conceivable that a substance could aid reversion to wild type—that is, repair—without being a carcinogen in animals. This seems more likely with frameshift mutants than with missense mutants.

Frameshift mutations generally involve intercalation of a planar molecule into the DNA base stack, thus generating a mispairing and frameshift when replication occurs (Bjeldanes and Chang, 1977). If equivalent planarity, a few free phenolic hydroxyls, and a modest size are all that are required, one could easily make a long list of natural phenols that should be active—ellagic acid and cyanidin, for example. Penetration to the genetic material may be another factor

of importance. To make quercetin soluble, dimethylsulfoxide was used in many of the experiments with *Salmonella*. A notable penetrant, it did not show activity with other flavonoids, but it is known to carry solutes into animal tissues. Penetration to the DNA of a prokaryote may not be the same as with a eukaryote, particularly with the barriers posed by the whole animal. Obviously, it is important to elucidate the true risk to humans and other animals of cancers from this source; one hopes that it will be proved as small a risk as now seems probable from the known exposure. At least it appears by the same sensitive Ames tests that most other flavonoids, including some synthetic ("abnormal") ones, are not mutagenic.

4. *Metabolism of Flavonoids and Further Consideration of Toxicity*

The metabolism of flavonoids by animals has been studied in some detail but is not completely understood (DeEds, 1968; Fairbairn, 1959; Farkas *et al.*, 1975, 1977; Griffiths and Barrow, 1972b; Kühnau, 1976; Mosser *et al.*, 1974; Williams, 1959). Many of the recent studies have used semisynthetic flavonoid derivatives. One of the early problems was that flavonoids were not detected in blood or urine after oral dosage, or were found only in traces after massive doses. It appeared that they were not absorbed. This view has now been corrected by both direct and indirect experiments. By circular dichroism, both free and protein-bound hydroxyethylrutosides could be detected in blood, reaching a maximum several hours after a 4-gm oral dose in man on a flavonoid-free diet and remaining detectable for 24 hr (Farkas *et al.*, 1977). By perfusion of the small intestine under conditions intended to avoid microbial effects, absorption of hydroxyethylrutin into arterial blood was found (Farkas *et al.*, 1975). In various studies of passive transport across model membranes and biological preparations, it has also been shown that absorption occurs, with the aglycones generally being absorbed most readily and the glycosides less readily. More was absorbed at acid (stomach) pH than at intestinal pH 6, and rutin was absorbed least, quercitrin and isoquercitrin more, and the aglycones (quercetin, kaempferol, myricetin, and rhamnetin) most (Kozjek *et al.*, 1975). Epicatechin-2-sulfonate was absorbed faster than catechin, and trihydroxyethylrutin least, in everted rat intestinal sacs; in all three cases the rate was increased by sodium taurocholate (Crevoisier *et al.*, 1975).

Among the reasons that absorption of flavonoids by animals is difficult to show by examining the blood and urine is the fact that the flavonoids appear mainly in the bile, particularly as glucuronides (Griffiths and Barrow, 1972b; Griffiths and Hackett, 1977; Hackett *et al.*, 1979). Another reason is the rapid disappearance of flavonoid from the blood by concentration in the liver and other tissues (Forster, 1977). Disappearance during perfusion of isolated rat liver was more rapid for the less water-soluble—that is, more nearly normal and less

substituted—members of the mono- through tetrasubstituted hydroxyethylrutin derivatives.

Still a third reason absorption of flavonoids is difficult to show is the appearance of only phenolic acid breakdown products in urine, so that it is not possible to say whether the flavonoid was absorbed intact or was broken down first. Oral administration of (+)-catechin, apigenin, myricetin, hesperidin, naringin, rutin, 3',4',7-tri-*O*- β -hydroxyethylrutoside, and 3',4',5,7-tetra-*O*- β -hydroxyethylrutoside to germ-free rats was followed by examination of the feces and urine for phenols (Farkas *et al.*, 1975; Griffiths and Barrow, 1972a). Phenolic acids, beyond a trace attributed to the diet, were not detected in the urine under circumstances in which they were found in rats with conventional intestinal flora. Conjugates of catechin and naringin were found in the urine from germ-free rats. Large amounts of unchanged rutin and the other glucosides were found in the feces, but no aglycones, corroborating previous reports that glycoside hydrolysis is the result of microbial action in the gut. Catechin was recovered unchanged from feces only in low amounts, indicating nearly complete absorption. Two catechin conjugates were found in both urine and feces in germ-free rats, but in neither in normal rats, indicating microbial breakdown of any conjugates reaching the feces via the bile in normal animals.

With the bile duct ligated, rats absorbed 91% of radioactive hesperetin (Brown *et al.*, 1978). The same authors were interested in preparing analogs of the highly sweet flavonoids that could be used for artificial sweetness in foods without being absorbable or metabolized by gut microflora. Sulfoalkyl derivatives produced by *O*-alkylation of the sweet dihydrochalcone with propane-1,3-sultone were sweet, poorly absorbed, and not degraded by microorganisms.

Enzymic extracts of animal organs are reported to break down flavonols to benzoic acids without the intervention of microorganisms (Kühnau, 1976). Perfusion of animal liver with apigenin gave six metabolic derivatives (Takacs and Gabor, 1975). Two of these appeared to be unidentified conjugates of apigenin, but the others were the 4-hydroxy derivatives of benzoic, cinnamic, phenylacetic, and phenylpropionic acid. Similar perfusion with rutin (Comèl and Laszt, 1972; Takacs and Gabor, 1975) gave eleven metabolites that were claimed, on the basis of paper chromatography and spectra, to include apigenin, luteolin, di- and trihydroxyflavone, isoferulic acid, 3,4-dihydroxyphenyl- γ -valerolactone, 3-methoxy-4-hydroxyphenylacetic acid, and 3-hydroxybenzoic, 3-hydroxyphenylacetic, and 3-hydroxyphenylpropionic acids. These studies indicate that, without microorganisms, animals can extensively metabolize flavonoids to acidic breakdown products similar to those produced by microorganisms, but in the normal circumstance, gut microflora play a large role.

The urinary constituents after oral administration of flavonoids to normal animals have been the most studied, but for the reasons just given the results may be somewhat misleading. Nevertheless, many data clearly indicate that the

flavonoids, by a combination of microbial and animal metabolism, are generally converted to a series of B-ring derivative acids, depending somewhat on the flavonoid (Farkas *et al.*, 1975, 1977; Kühnau, 1976). Different animals give generally similar products, and this may, in part, be due to similar microflora. Flavonoid pigments have been reported to be excreted without change in normal carnivore urine, but not in the urine of herbivores (Bate-Smith, 1954). The A-ring portion of the flavonoid is generally metabolized more or less completely to CO_2 , and as a rule the B-ring acids appear as mixtures of benzoic, phenylacetic, and phenylpropionic acid derivatives, depending on the degree of oxidation and the original flavonoid (Kühnau, 1976).

Catechin, which has been the most completely studied, appears unique in that, in addition to the expected hydroxyphenylpropionic and hydroxybenzoic acids, there was a series of hydroxylated and methylated neutral compounds, phenyl- γ -valerolactones. These lactones are apparently produced by oxidation and fission of the catechin A-ring to oxalacetic acid and a δ -lactone from the heterocyclic ring with the B-ring attached (Fig. 4). This substance is analogous to antibiotically active natural products. The δ -lactone is unstable and rearranges to the phenyl- γ -valerolactone (Fig. 4). In men receiving 4.2-gm oral doses of catechin, no effects were noted or slight diarrhea; 7.5% was excreted unchanged in the urine, and 18.6% in the feces (Das, 1971). Of eleven urinary metabolites, the major ones were 3-hydroxyphenylpropionic acid, δ -(3,4-dihydroxyphenyl)- γ -valerolactone, and δ -(3-hydroxyphenyl)- γ -valerolactone. Sulfate and glucuronide conjugates of the catechin and of the three metabolites were also found. A small amount of δ -(3-methoxy-4-hydroxyphenyl)- γ -valerolactone glucuronoside was also observed.

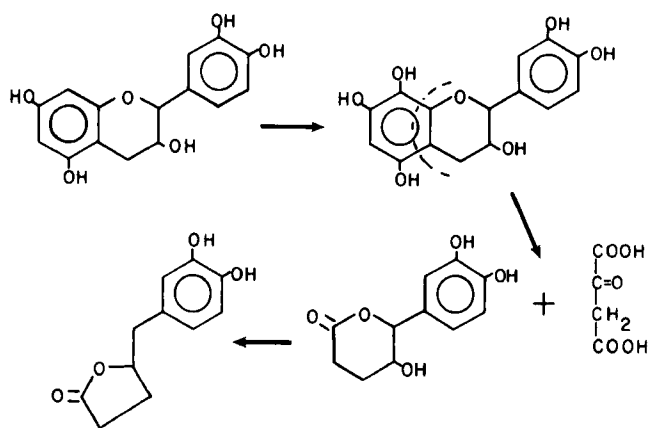


FIG. 4. Probable route of conversion of (+)-catechin to γ -valerolactone derivatives by intestinal flora of animals.

Catechin's breakdown in the rat was similar to that in man, but in the guinea pig and rabbit the products were fewer, and the major ones (3-hydroxybenzoic, vanillic, and protocatechuic acids) were not detected in human urine. In the monkey, oral or intraperitoneal administration of radioactive catechin resulted in urinary excretion of 53.5 and 50.2%, respectively, of the dose (Das, 1974). Radioactivity in the feces was 0.8–2.2% of the dose; 4.3% was excreted as 3-hydroxyphenylhydracrylic acid, and 7.6% as δ -(3-hydroxyphenyl)- γ -valerolactone, the two major metabolites. The hydracrylic acid arose from β -hydroxylation of 3-hydroxyphenylpropionic acid.

Considering that a large part of the flavonoids is excreted via the bile (Griffiths and Barrow, 1972b; Griffiths and Hackett, 1977; Hackett *et al.*, 1979), it is perhaps reasonable that oral doses of about 100 mg/kg (and hydroxycinnamates likewise) have been reported to stimulate bile flow, lower the blood cholesterol, and help protect against an atherogenic diet (Kiyasheva, 1974; Lisevitskaya *et al.*, 1970). Such an effect from the phenols of red wine (Masquelier, 1961) appears to be variable in rats, depending on their sex and history (Stoewsand and Anderson, 1974). A flavonoid preparation from lespedeza and robinin, a kaempferol glycoside, has been reported to increase the flow of urine and urea output, and to improve experimental renal insufficiency (Berger, 1968; Drumev and Pashov, 1970; Gulyaev, 1972).

Protective effects of flavonoids against other xenobiotics by induction of liver microsomal mixed-function oxidases and by other mechanisms have been reported, but mostly by derivatives not known or rare in nature. Flavone and methoxyl (but not hydroxylated) derivatives induce a two- to threefold increase in lung and liver benzyrene hydroxylase activity (Cutroneo *et al.*, 1972; Wattenberg *et al.*, 1968). Tangeretin, however, was reported to be inactive.

Various flavonoids and semisynthetic modifications have been found able to minimize experimental damage to the liver from such agents as carbon tetrachloride (Heintz *et al.*, 1963). This is probably due to free radical scavenging, because the trichloromethyl radical is believed to be the toxic form of carbon tetrachloride activated by cytochrome-450. Vitamin E, for example, also hastens recovery from the injury. Catechin *in vitro* (and the antioxidant propyl gallate) strongly inhibited the lipid peroxidation system involving carbon tetrachloride, but *in vivo* no protective action was found (Danni *et al.*, 1977). Catechin as a treatment for hepatic diseases has undergone clinical trials involving 1000 patients at forty-eight centers in eleven European countries (Alhadeff, 1977). Tolerance at a typical dosage of 1.5 gm/day *per os* was excellent, with slight gastrointestinal side effects and "no known contraindications." A prophylactic as well as therapeutic effect was claimed in treating partial hepatectomy, effects of alcohol on the liver, liver lesions from prolonged isoniazid, acute viral hepatitis, and toxic hepatitis. A favorable effect in restoring bilirubin levels to normal in alcoholic hepatitis was noted, as well as the effect in experimental animals of

increasing liver ATP and preventing the fall in ATP due to toxic agents, such as malonate.

Anthocyanin preparations from red peanut skins and cashews caused goiter (increased thyroid weight) in rats in 7 weeks when fed at 20 mg/day (Jeney, 1968; Moudgal *et al.*, 1958). The effect was countered by the addition of extra iodine to the diet. It appears that phloroglucinol, all flavonoids containing it, and any other aromatic group having electron-donating groups and capable of forming iodine substitutions are weak goitrogens. They apparently compete for the available iodine, and a significant amount of iodine appeared in the phenolic fraction of excreta. This effect might well contribute to the seriousness of the absence of adequate iodine, but the activity is mild compared with that of other types of goitrogens.

A toxic effect of flavonoids that probably has benefited man is their inhibitory action on microbes and viruses. The use of tea or wine as a preventive for certain diseases has a long history. It has long been known that these effects are due to the phenolic compounds and not to other constituents, such as the alcohol in wine. They are weak compared with the effects of commercial antibiotics, but they are sufficient to excite new scientific interest periodically, as reported in the public press. Some effects appear to be general in the phenols (Singleton and Kratzer, 1969), and the flavonoids are no exception. In tea, it is the catechins, particularly epigallocatechin and its gallate ester, that are active, green tea infusion being more so than black (Das, 1962). Naringenin and hesperitin are the most active flavonones. Flavonols are active, especially morin (unusual 2'-hydroxylation), and dihydroflavonols are more active than the corresponding flavonol (Ramaswamy *et al.*, 1972).

In wine and related products, attention has centered on anthocyanins, but polymerization to form tannins seems also to be involved, since activity increases for a time as wine ages (Fairbairn, 1959; Masquelier and Jensen, 1953). In general, pyogenic and enteric bacteria of the gram-positive group are most susceptible to the phenols and flavonoids (Das, 1962; Lucia, 1963; Pagenkopf and Woodburn, 1970; Powers *et al.*, 1960; Singleton and Kratzer, 1969). Quercetin, taxifolin, and red wine flavonoids are able to inactivate a wide range of viruses and prevent their infectivity (Bakay *et al.*, 1968; Konowalchuk and Speirs, 1976). Although these activities are puny in a therapeutic sense, historically they were very significant in a preventive sense. Wine was used as an important part of the provisions of the hugely successful Roman armies. Even during our Civil War, the largest loss of life was due to poor sanitation and enteric diseases, such as dysentery, typhoid, and viral hepatitis. Mixing wine and its flavonoids with water and allowing time for the water-borne pathogens to be killed was a treatment well known to the ancients and to the moderns in the Mediterranean area (Konowalchuk and Speirs, 1976; Lucia, 1963). It has been shown by experiment that these pathogens do not survive long in such mixtures. Many of our

forebears probably survived because of flavonoids in wine, and if wine had been available and appreciated in the 1860s, the Civil War might have been less bloody.

Anthocyanins have been reported to have a favorable effect on vision in man as well as in experimental animals, particularly with regard to regeneration of visual purple and rapid adaptation to the dark (Alfieri and Sole, 1966; Wegmann *et al.*, 1969). Anthocyanin preparations from myrtle, cassis, and red wine grapes have been used on 8000 patients in Europe, with 73–97% improvement in various vascular conditions of the bioflavonoid-responsive type; these preparations have also been given for 15 years to improve night vision and myopia retinopathy (Pourrat, 1977). The typical dose was 600 mg/day by mouth. Little difference was seen among the various anthocyanins, and tolerance was no problem.

If the usual flavonoids are generally low in toxicity, are the less common forms more toxic? The answer appears to be typically, but not invariably, yes, and the more unusual the flavonoid, the more toxic it is. A host of semisynthetic derivatives have been tried for various conditions, and some of them appear to have special activity (Bezanger-Beauquesne, 1977; Bogнар and Rakosi, 1977). Many of them, including the tris-3',4',7-(β -hydroxyethyl)rutin previously mentioned, involve relatively minor additions to the basic natural flavonoid structure. The toxicities have generally remained fairly low for these compounds—some are similar to that of the parent natural compound. Some have additional or intensified physiological effects. Dimeflin, 3-methyl-7-methoxy-8-dimethylaminomethylflavone (Fig. 5), for example, is a powerful respiratory stimulant with an effect in humans after an oral dose of the order of 24 mg, and a minimum lethal dose in rats of about 8 mg/kg given intravenously (Scanni *et al.*, 1977; Setnikar and Magestretti, 1964).

Unusual flavonoids of natural occurrence are of more interest here. Tangeretin (Fig. 3) and nobiletin are completely methoxylated derivatives of kaempferol and quercetin, respectively, with additional methoxyls in positions 6 and 8. This type of compound is incapable of glycoside formation and is found mainly in the peel oil sacs, with distribution limited primarily to certain citrus fruits and a few essential oils. These and related compounds have strong antimicrobial and antiviral activity, and they are part of the plant's natural resistance to certain of its diseases (Kühnau, 1976). Compounds of this series have been found to be cytotoxic in a zebra fish embryo assay, when the usual flavonols were not (Jones *et al.*, 1964). Although not toxic to adult mice when given intraperitoneally or subcutaneously at 450 mg/kg, or to rats or dogs, tangeretin caused 83% of the offspring to be born dead or to die within 3 days when 10 mg/kg per day was given subcutaneously in carboxymethylcellulose to pregnant female rats (Singleton and Kratzer, 1969, 1973; Stout *et al.*, 1964).

Diosmin, with one more methoxyl and two fewer hydroxyls than rutin, has bioflavonoid activity and was nontoxic at 60–620 mg/kg per day given orally

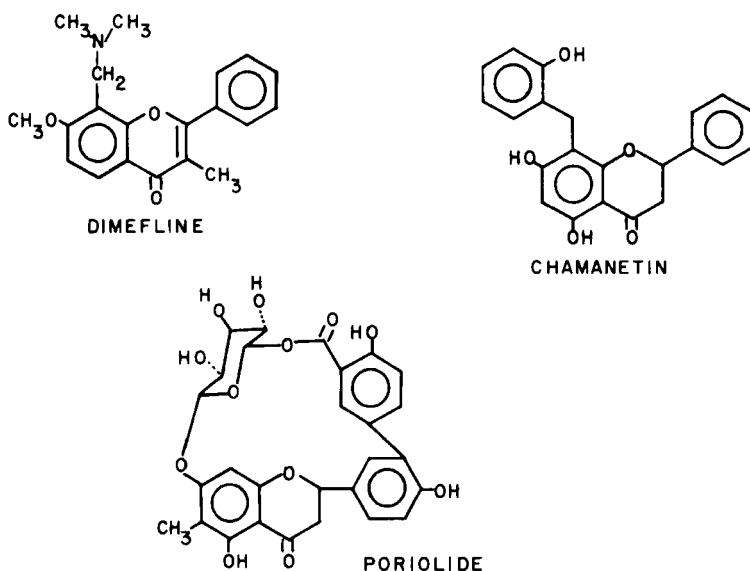


FIG. 5. Examples of unusual and physiologically potent or toxic flavonoids.

to dogs (Heusser and Osswald, 1977). Polygalactin, 3-rutiosyl-3,7-dimethylquercetin, is also considered to be a bioflavonoid that is low in toxicity, but it shows antimutagenic activity in onion cells (Reddy *et al.*, 1977). A series of cytotoxic flavones, also not glycosides and 6- or 8-methoxylated, have been isolated from *Eupatorium* species (Kühnau, 1976) and *Lychnophora affinis* (LeQuesne *et al.*, 1976). They show activity against cell cultures of human nasopharynx carcinoma and in *in vitro* transplants. It appears from this series that lack of glycosidation (which would affect absorption and microbial breakdown in the gut), decreasing the phenolic character by progressive methoxylation, and further substituting the A-ring by methoxyls, all tend to increase the toxicity. However, it is also clear that the maximum acute toxicity of this series, as represented by nobiletin, is not high. Embryos with not only rapidly multiplying cells, but also weak defenses against toxins metabolized in the liver, seem to have the only significant vulnerability.

Rather high acute toxicity is possible in the more unusual flavonoids from nonfood plants, such as chamanetin (Fig. 5) and its 6-benzyl analog isochamanetin from *Uvaria chamae* (Lasswell and Hufford, 1977), and poriolide (Fig. 5) and isoporiolide from *Leucothoe keiskei* (Ogiso *et al.*, 1977). The latter pair had an LD₅₀ in mice of 1.0 and 1.6 mg/kg, respectively, when given intravenously. Rotenone, a botanically rare and complex isoflavonoid, is useful because of its toxicity (Singleton and Kratzer, 1969, 1973). Isoflavonoids, in general, are more

toxic or disruptive to animals, several acting as estrogens (Harborne, 1979; Singleton and Kratzer, 1969, 1973). However, rather unusual flavonoids—the silymarin constituents, for example (Cavallini *et al.*, 1978; Faulstich *et al.*, 1980)—appear to be antitoxic.

F. TANNINS

The primary role of tannins in plants appears to be as a defense against attack by bacteria, fungi, or herbivores (Swain, 1979). Tannins have been responsible for outbreaks of fatal poisoning of domestic animals of considerable local commercial importance, particularly with regard to grazing of *Quercus havardi* in the American Southwest and acorn consumption in Europe, when food was so restricted that little else was available to eat. Toxic or deleterious properties associated with excessive feeding of carob, lespedeza, certain sorghums, rapeseed oil meal, grape seed meal, and other high-tannin products are known. Human fatalities have been caused by treatment with tannic acid in enemas or on burned skin. Before reviewing these manifestations of tannin's toxic potential, let us consider different classes of tannins and some of their collective and differential properties.

In regard to their chemical behavior, leather-tanning properties, astringent flavor, and physiological effect, tannins have in common a polyphenolic macromolecular nature, which binds with protein to insolubilize it. It is only fairly recently that specific molecular forms of true tannins have been separated and characterized (Haslam, 1966, 1975). Highly purified and well-characterized pure tannins seldom have been available for animal testing, especially not in the amounts necessary for feeding trials. Many of the data on the toxicity and physiological effects of tannins have been obtained on mixtures that, even when free of nonphenols, contain components representing a considerable range of molecular weight and isomerism. This is not necessarily a serious problem, since the physiological effect of an octamer, say, is not likely to be drastically different from that of a heptamer. The effects at the extreme should be considered, however, and also the considerable difference in the type of phenolic "monomers" making up tannins of different classes—specifically hydrolyzable gallo- and ellagitannins vs condensed flavonoid (and, more rarely, stilbene, etc.) tannins.

The minimum molecular weight is about 350 for effective protein precipitation as measured with gelatin, for example. In the condensed series this would begin with dimeric flavonoids. In the hydrolyzable series no fewer than two gallic acid precursor units or one ellagic acid precursor unit would be the theoretical minimum. When the molecular weight is quite large, probably more than 5000, the condensed tannins, particularly, become so poorly soluble in physiological solutions, even in the absence of protein, that they have little leather-forming ability or astringent taste. They also seem relatively inert physiologically, espe-

cially when modified by heat, etc., into "phlobaphenes," unless injected, and even then they appear to act mainly as inert, obstructive particles. Phlobaphenes are poorly characterized, additionally polymerized, partly oxidized, and very poorly soluble phenolic macromolecules such as are found in outer tree bark. For our purposes, we shall generally accept Haslam's description of "tannin" as valid, representing a fairly water-soluble, natural polyphenolic mixture of intermediate molecular weight.

Since all tannins are polyphenols and function as protein binders and precipitants, their chemical and physiological properties are somewhat similar. Many authors speak of "tannin" as if it were a single entity. Other poor terminology can confuse readers, particularly that found in the older literature. Digallic or *m*-digallic acid is a subunit of gallotannic acids, but it has never been readily available, and tannic acid has been sometimes so misnamed. Tannic acid has frequently been used incorrectly as a general term for tannins. Tannic acid should refer only to specific hydrolyzable gallotannins, but they have been the ones most commonly used in testing biological effects.

Tannic acid of commerce has generally been Chinese (from *Rhus semialata* leaf galls) or Turkish (Aleppo tannin, from leaf galls of *Quercus infectoria*), both of which are predominantly glucose esterified with 6 to 9 gallic acid units. Tara "tannic acid" (from pods of *Caesalpinia spinosa* of South America) has 4 or 5 gallic acid units esterified to quinic acid. Various similar gallotannins are prepared from other plants, notably the tropical fruits called myrobalans, maple, witch hazel, chestnut wood, and oak wood (Haslam, 1966). Ellagitannins also come from myrobalans, valonea (acorn cups of *Quercus valonea*), divi-divi, algarobilla (other *Caesalpinia* species), and walnuts (Haslam, 1966). Except for their different hydrolytic products—gallic and ellagic acids, which have already been discussed—the gallotannins and the ellagitannins are quite similar. They frequently occur together, and many ellagitannins also contain gallate units.

The condensed tannins of commerce include quebracho (from heartwood of a South American tree), wattle (wood and bark of certain Acacias), and mangrove bark extracts. The properties of the dimeric flavans, the smallest condensed tannins, bear considerable resemblance to the properties of their monomers—catechin and related bioflavonoids, already discussed. Oligomeric condensed tannins serve as bioflavonoids in increasing the weight gained by guinea pigs on minimal ascorbic acid regimes and extending their survival (e.g., Laparra *et al.*, 1978). Monomers, however, whether catechins or gallate, may bind to proteins by similar hydrogen-bonding mechanisms, but ordinarily they do not precipitate proteins such as gelatin. This is a fundamental difference between tannins and their component phenols, but it is somewhat a matter of degree. Sometimes hydrolyzable and condensed tannins are produced in the same plant, but generally in separate tissues. Oak and chestnut, for example, produce hydrolyzable tannins in wood and new growth, but condensed tannins in outer bark (Haslam, 1966).

Hydrolyzable tannins are relatively uncommon in human food, but they do occur in the brown outer skin of walnut meats and probably other nuts, and in leachates from barrels used for beverage storage, and pharmaceutical-grade tannic acid has been added to foods and drugs. Condensed tannins are widespread in fruits and in certain grains, and are high in some less common vegetable foods—grape leaves, for example (Haslam, 1975; Hermann, 1959). Their classification as hydrolyzable or condensed in a poorly studied food often depends on whether or not cyanidin or related anthocyanidins are generated when the astringent tissue or its alcoholic extract is boiled with mineral acid. Astringent and bright red means proanthocyanidin, anthocyanogenic condensed tannin, and astringent without reddening indicates hydrolyzable tannin. Chromatographic identification of cyanidin in the former case and gallic and/or ellagic acid in the latter is considered to be confirmation.

The amount of tannin in plant material used commercially as sources is about 5-50% of the dry weight, with about 20% as the usual amount. Nondescript herbage frequently has 1-5% tannin. As was discussed briefly earlier, animals and humans generally avoid consuming appreciable amounts of high-tannin materials (their astringent flavor being a major reason). A reasonably high astringency may be accepted by animals when access to other food is prevented. Under such circumstances, animals may eat materials with 20% tannin, but toxic effects result, as will be discussed shortly. Animals may accept by preference occasional bites of higher-tannin herbage, even when low-tannin food is available. Man consumes a number of foods containing considerable amounts of condensed tannins, especially in beverages such as cider, cocoa, tea, and red wine. Strong versions of these beverages may have as much as a gram of tannin per liter. An adequate level of tannin in these products and some other foods, such as spinach, stuffed grape leaves, persimmons, and bananas, is considered desirable. The intake of dimeric flavans has already been indicated as about 400 mg/day in human diets (Kühnau, 1976). Total tannin intake would be somewhat higher. Per capita consumption of red wines in some countries would guarantee an average intake of nearly that amount from this source alone. Certain consumers may take in severalfold the average consumption.

Since they are uniquely potent binders and precipitators of protein, it is not surprising that tannins are toxic when injected into the blood stream and that they have inhibitory effects on enzymes and microorganisms. It is well known among plant biochemists that, in order to isolate active enzymes from higher plants in good yield, routine competitive binding of the omnipresent tannins is necessary. Periodically reports appear of the tracking of potent enzyme inhibitors in plant material back to tannins. Enzymic activity may still be demonstrable if an enzyme-tannin precipitate is resuspended in substrate solutions, but usually it is considerably reduced. For similar reasons it is the general finding, and clearly a logical one, that tannins can bind to viruses and microorganisms to inhibit their

activities. Since phenols in general have antimicrobial properties and tannins have the additional property of binding to proteinaceous surfaces, etc., a rather general but nonspecific "antibiotic" effect by tannins is to be anticipated. The role of high tannin content in helping to protect some plants against pathogens is generally accepted.

Many articles could be cited describing antibiotic, antiviral, anticellular, antineoplastic, and related effects of tannins, most *in vitro*, but a few examples will suffice (Alexander, 1965; Chan *et al.*, 1978; DeOliveira *et al.*, 1972; Herz and Kaplan, 1968; Konowalchuk and Speirs, 1976; Kreber and Einhellig, 1972; Loub *et al.*, 1973). The effect of tannin on microbes can have a dietary influence in animals. *In vitro* or in fistulated animals the microorganisms of the rumen can be inhibited completely by addition of 7.5% or more tannic acid. Phosphate uptake and synthesis of nucleic acids and proteins by the microorganisms were inhibited proportionally by lesser concentrations of Tannin (Sadanandan and Arora, 1976). The breakdown of peanut protein by goat rumen fluid was inhibited 62 and 87% respectively, by addition of 2 or 4 gm of tannic acid per liter (Tripathi, 1978). Field beans with colored flowers contained 4-8% tannins in the testa, versus less than 0.6% in white-flowered varieties. Among 24 bean varieties, the cellulase digestibility of the testa was negatively correlated with tannin content (Griffiths and Jones, 1977).

This type of microbial inhibition may have negative or positive effects on the animal, depending on the situation. Microorganism-dependent cellulose digestion is inhibited, and utilization of poor-quality feed by the ruminant is impaired. Sheep fed sorghum silage with 18.7 gm of tannin per kilogram had depressed digestion of crude fiber and less microorganism activity in the rumen when compared with sheep fed maize silage containing 6.6 gm/kg (Ben-Ghedalia and Tagari, 1977). Sometimes, however, the animal can benefit from the minimizing of microbial competition for starch and amino acids (Hatfield, 1970). Treatment of soybean meal with 10% tara tannic acid reduced deamination of the meal by rumen fluid by 90%, but improved the average daily gain and nitrogen balance in lambs (Driedger and Hatfield, 1972). Other tannins had similar effects.

Also in ruminants, bloat (a sometimes fatal condition of high rumen pressure) is evidently prevented by sufficient tannin in the feed. The foaming, which causes pressure retention, is prevented by precipitation with tannins of the proteins, which otherwise form stable foam (Jones *et al.*, 1973). The production of gas by microbial fermentation is also inhibited. The site of nitrogen metabolism is transferred from the rumen to the intestine of sheep by feeding sainfoin with condensed tannins, owing to the formation of protein-tannin complexes that are stable in the rumen (pH 6.5) but not in the abomasum-duodenum (pH 2.5) (Jones and Mangan, 1977).

Still another microbial effect with dietary significance may appear in monogastric animals and perhaps in ruminants as well. Tannins at an appropriate

level apparently can suppress detrimental microflora in the alimentary tract and stimulate growth and good health; the effect is similar to that seen after more potent antibiotics are given (Produits Chimiques et Celluloses Rey, 1964).

The acute toxicity of tannins given orally to animals is low, but, as would be predicted for a protein precipitant, it is much greater when they are given parenterally. The LD₅₀ values for mice, rats, and rabbits after a single large dose of tannic acid given orally are generally between 2.25 and 6 gm/kg body weight (Boyd and Boyd, 1973; Singleton and Kratzer, 1969, 1973). Acute LD₅₀ values for rectal administration are significantly lower—often about one-half. The LD₅₀ for intraperitoneal or subcutaneous administration in rats is of the order of 200–700 mg/kg, and for intravenous administration, about 20–80 mg/kg (Singleton and Kratzer, 1969). A condensed tannin preparation from hawthorn with small flavonoids removed gave an intraperitoneal LD₅₀ of 130 mg/kg in mice and a subcutaneous LD₅₀ of 300 mg/kg (Rewerski and Lewak, 1967). A condensed tannin from grapes was fatal to rats at 150–200 mg/kg given intraperitoneally, and 75 mg/kg given intravenously killed rabbits (Patay *et al.*, 1962). In drinking water 1 gm/liter caused the death of some animals by 19 days. Gastric irritation and hepatic lesions were present at autopsy.

When sufficient tannin is injected intravenously, a sudden fall in blood pressure occurs, and death resembles, but can be distinguished from, anaphylactic shock (lack of histamine production, bronchiospasm rather than cardiac arrest) (Fiedler and Hildebrand, 1954; Hildebrand, 1958).

A large condensed tannin from lespedeza (14,000–20,000 MW) and a butanol-soluble, oligomeric, condensed tannin from grapes, fed for 120 days and up to 700 days, respectively, to rats at 2% in the diet, produced no detected gross or histopathological effects (Booth and Bell, 1968). Similarly, no toxic effects were found in rats fed tannin at 1000 mg/kg per day for 231 days (Blumenberg and Kessler, 1960). However, in other studies with rats and other animals, 1–5% tannin of various types in the diet was frequently inhibitory to growth or was otherwise toxic. Blakeslee and Williams (1979), with 1% tannic acid in the diet of laying hens for 6 wk, found no significant differences in egg production, feed efficiency, or fertility or hatchability of the eggs, although feed consumption was reduced in the second and third weeks. Tannic acid at 2 or 4% decreased feed consumption and egg production. Salseed at 50 gm/kg of diet (about 0.6% dietary tannin) or more gave depressed chick growth and feed conversion (Zombade *et al.*, 1979). The threshold of toxicity of tannic acid added directly to rumen contents in fistulated animals was 3–5% in cattle but 8–10% in goats, apparently because the goat produced an active tannase in the rumen mucosa (Begovic *et al.*, 1978a).

Condensed tannins tend to be more inhibitory to enzymes *in vitro* than does tannic acid (Tamir and Alumot, 1969). Tea tannin (condensed) was toxic at 1%, but Chinese tannic acid at only 0.8%, in experiments with worms (Tkabladze,

1959). Neither type of tannin added as 10% of the diet affected generally feeding grasshoppers, but hydrolyzable and not condensed tannin was deleterious to a graminivorous locust (Bernays, 1978). The hydrolyzable tannin passed through the peritrophic membrane and damaged the epithelia of the gut. Depression of growth in weanling rats was greater after gallotannin was fed than it was after condensed tannins were given (Joslyn and Glick, 1969). Ellagic acid had no effect on the rats or on chick growth (Kratzer *et al.*, 1975). On a weight basis, all other phenols tried on the chicks, except methylgallate, were less growth-inhibitory than were two samples of tannic acid. On a phenol content basis, two ellagitannins, two gallotannins, and two condensed tannins (eucalyptus and wattle) had essentially similar effects (98–117% of tannic acid's inhibition), but tara tannic acid and the condensed tannins from mangrove and quebracho were less toxic—76, 72, and 39%, respectively (Kratzer *et al.*, 1975). The differences could not be satisfactorily accounted for by relative dialyzability, but there was a tendency for the less dialyzable, larger molecules to be less inhibitory. Tannic acid was more toxic and growth-depressing for chicks than were condensed and other hydrolyzable tannins in other tests (Lapaz *et al.*, 1975; Vohra *et al.*, 1966). It is apparently a matter of relative size, perhaps gut pH, firmness of binding to proteins and ease of partial breakdown as to which tannin is more inhibitory.

Condensed tannins were much less toxic than hydrolyzable tannins when used in burn therapy (Wilson and Gisvold, 1956). Subcutaneous injection of condensed tannins produced only local damage, but hydrolyzable tannin produced liver damage as well (Kirby, 1960).

The negative results of feeding tannin in large amounts are attributable to the combined effects of several causes in addition to the microbiological ones already mentioned. The astringent flavor makes the diet unpalatable, and an insufficient amount is eaten for maximum growth rate in the young or, less often, for good health in the adult animal. Protein in the diet is bound, and therefore it passes through the animal without being utilized. Digestive enzymes (proteins) are precipitated and prevented from properly digesting the food. In response to inadequate enzymic digestion and the denuding of mucoproteins from the surface of the gut, extra quantities of these materials are synthesized by the animal, thus placing an extra load on its nutritional status and health. All these factors can be involved without a direct toxic effect of the tannin or actual entrance of tannin into the tissues. Direct irritant or toxic effects at the surface or within the body are additional reactions to tannin toxicity. Breakdown products of the tannins, flavonoids, or particularly gallic acid, although low in systemic toxicity, can be produced in sizable amounts.

The role of astringency in causing waste because the diet is unpalatable is sizable. It can be estimated by paired feeding—keeping the weight of the intake of the animals on the control diet the same as the weight of the food consumed by those on the diet with tannin added. A considerable portion, but generally not all, of the inhibitory effects of tannin is accounted for by such paired feeding of rats

(Glick and Joslyn, 1970a; Handler and Baker, 1944; Mueller, 1942) or chicks (Vohra *et al.*, 1966). It does not seem fair to consider this effect as "toxicity," since it is the result of a failure to consume rather than the result of consumption. The effect seems to be larger in young animals (Glick and Joslyn, 1970a), partly because of their proportionally larger dietary needs, but also because they may be less tolerant of unpalatable diets.

Much of the remaining deleterious effect of tannin in the diet, once it is actually consumed, is related to tannin's binding of the dietary protein, thus making the diet inadequate (Vohra *et al.*, 1966). Once formed, most protein-tannin complexes are difficult to break down, and they pass out in the feces. The nitrogen content of the feces generally rises in proportion to the amount of tannin fed. Carob pods, when green, contain partly hydrolyzable tannins at about 200 gm/kg dry weight and, when ripe, about 70 gm/kg dry weight of condensed tannins (Joslyn *et al.*, 1968). Although the ripe pods seemed to be reasonably palatable to rats and food intake was not much lowered, protein was bound, fecal nitrogen went up, and treatment of the feces with urea released phenol from protein binding (Tamir and Alumot, 1970). Finger millet has a tannin content of about 0.05% for the white-grained varieties and up to 2.5% for brown-grained varieties. Protein digestibility *in vitro* decreased in proportion to an increase in tannin content or with addition of tannic acid to the low-tannin grain (Ramachandra *et al.*, 1977). The tannin content of barley samples, 0.55–1.23%, correlated negatively with protein utilization when the barley was fed to rats (Eggum and Christensen, 1975). The availability of all amino acids declined, but to different degrees. Barley of the same variety grown under carefully matched fertilization schedules had 0.18–0.75% tannin, depending on the northerly (high) or southerly (low) location of the plots (Gohl and Thomke, 1976).

Selections of sorghum are bird-resistant or bird-proof because wild birds do not relish their high tannin. They are also more resistant to bad weather and preharvest germination. The red, high-tannin varieties may contain 7–8% condensed tannin, while the whiter, low-tannin varieties have less than 1% (Mab-bayad and Tipton, 1975; Martin-Tanguy *et al.*, 1976). The bird-proof types are important, particularly in semiarid areas where competition for food is severe. Sorghum is considered the fourth most important grain for human consumption, and it is primary in dry areas of Africa and Asia. Again, protein and energy digestibility is reduced for rats and chicks fed sorghum with increased tannin content (Featherston and Rogler, 1975). Extraction of the tannin improved the food value, and addition of the extracted tannin to low-tannin grain lowered it. Treatment of high-tannin sorghum grain for several days with water or, better yet, with alkaline solutions of ammonia, potassium carbonate, or sodium bicarbonate decreased the extractable tannin about 50–99%. The treated grain could be nutritionally equivalent, or nearly so, to low-tannin sorghum for rats and chicks (Price *et al.*, 1978, 1979; Reichert *et al.*, 1980).

Simple conversion of high-tannin sorghum grain to a batter or cooking the

grain, even though the extractable tannin was considerably reduced, did not improve weight gain and even lowered it somewhat, compared with results obtained with untreated grain (Price *et al.*, 1980). Differences produced in the tannins, perhaps by rapid oxidative polymerization in alkaline solution as well as by hydrolysis, and in the extent and firmness of protein complexing appear to be involved. Cooking low-tannin sorghum did not lower its nutritional quality. Tannic acid treated with oxygen in alkaline solution for 24 hours and fed for 25 days to young rats (Mitjavila *et al.*, 1978) depressed rat growth, but in comparison with untreated tannic acid, it decreased the nitrogen excreted in the feces and increased that in the urine.

Field or horse beans (*Vicia faba*) with high condensed tannin and the tannin separated from them depress the growth of ducklings, the laying rate of hens, and protein digestibility in chicks (Griffiths and Jones, 1977; Guillaume and Bellec, 1977; Guillaume and Gomez, 1977; Martin-Tanguy *et al.*, 1977). Similar effects are also found with common dry beans (Ronnenkamp, 1978). The tannins are concentrated in the seed coat (as is true for most other seeds) and are of the order of 40 gm/kg of seed coat with several molecular sizes present (Martin-Tanguy *et al.*, 1977). In feeding chicks low-tannin vs high-tannin varieties of fababeans and control diets, the tannin accounted for at least 50% of the growth depression from fababeans. The tannin level in the diet was highly correlated with decreased feed intake, decreased weight gain, increased feed per unit gain, increased fat retention, and decreased retention of protein, dry matter, calcium, and ash (Marquardt and Ward, 1979). Autoclaving the beans gave some improvement in feeding value whether or not tannin was present, suggesting that additional factors are involved.

If binding dietary protein were the only direct effect of dietary tannins, then supplementation of the diet with extra protein should eliminate it. Protein supplementation does markedly alleviate the growth-depressing effect of tannic acid (Glick and Joslyn, 1970b). If extra casein was added to a 5% tannic acid diet for rats, the fecal nitrogen was no higher than that found in rats given a diet without the casein, indicating complete utilization of the extra casein. In rats, eating tannic acid at 50 gm/kg of diet, plus protein at 60 gm/kg, gave the maximum amount of fecal nitrogen (Mitjavila, 1973; Mitjavila *et al.*, 1974). The indication that about equal weights of tannin and protein are bound agrees with chemical studies. Increased nitrogen and dry weight of feces were produced with increased tannic acid. There was less body weight loss when 6% protein was added in the tannic diet, but it still occurred, whereas without tannic acid it did not.

When the tannic acid was oxidized before it was fed to rats, it gave lower fecal nitrogen retention and had less severe effects on the animals (Mitjavila *et al.*, 1978). Oxidation converts hydrogen bond donor hydroxyls into acceptor quinone carbonyls. This decreases protein binding by the usual tannin mechanism, but it

may give substitution reactions with lysine amino or cysteine sulfhydryl groups. Since phenol oxidation is greatly accelerated at alkaline pH, this is probably the reason the addition of calcium hydroxide or sodium carbonate improved the growth of chicks fed tannic acid (Rayudu *et al.*, 1970b). Tannin-binding agents poly(vinylpyrrolidinone) and polysorbate 80 also improved growth.

In general, it appears that amino acid supplementation to overcome dietary tannin inhibition is about as effective as the protein for which it substitutes, although relatively little experimentation has been done, except for methionine. Methionine holds a special place for diets containing mainly legume protein, because it is the most deficient amino acid in legume protein for animal nutrition. It is also considered to have a special role in detoxicative methylation of tannin breakdown products, notably gallic acid and the phenolic acids from condensed tannin flavonoid fragments. Supplementation of tannin-containing diets with the methyl donors methionine and choline has often counteracted a major part of the effects of dietary tannins, particularly with chicks and with both condensed and hydrolyzable tannin (Armstrong *et al.*, 1974; Featherston and Rogler, 1975; Fuller *et al.*, 1967; Guillaume and Bellec, 1977; Rayudu *et al.*, 1970a; Vohra *et al.*, 1966).

That enzyme inhibition is also involved in dietary tannin's inhibition of animal growth is indicated by the general finding of inhibition not only of protein digestion, but also of the digestive yield of metabolizable energy (Featherston and Rogler, 1975; Gohl and Thomke, 1976; Griffiths and Jones, 1977). Rapeseed meal has about 3% tannin; extraction of this tannin increased the available metabolizable energy content for 2-week-old chicks from 1171 kcal/kg with tannin to 1844 kcal/kg without (Yapar and Clandinin, 1972). Admission of 0.5% tannin solution to the true stomach of calves decreased the pepsin activity to one-half to one-third, and the rennet activity to one-fifth to one-fifteenth (Lebedev, 1969). A three- to fourfold increase in total production of intestinal proteolytic enzymes was noted in rats fed tannic acid (Glick and Joslyn, 1970b). Griffiths and Moseley (1980) found that the tannin of field bean testa inhibited trypsin and amylase activity in the intestine of rats, but increased lipase activity. The tannin stimulated pancreatic secretion, and the lipase had less affinity with the tannin than did the other enzymes. Treatment of the intestinal contents of tannin-fed rats with polyvinylpyrrolidone-saline extraction resulted in a recovery of trypsin activity similar to that found with the low-tannin diet, showing that trypsin-tannin binding was the major factor.

This high level of enzyme production after binding with tannin can contribute to the high nitrogen content of the feces. Tannin consumption causes a loss in body weight, a loss of nitrogen in the feces in proportion to body weight (Mitjavila *et al.*, 1974), and sometimes a greater total excretion of nitrogen in the feces than is taken in in the diet; it is clear that not just dietary protein is involved in tannin binding. The increased excretion of mucoprotein, sialic acid, and

glucosamine in the feces in the presence of tannic acid (but less with oxidized tannic acid) fed to rats indicates that mucus hypersecretion is the main source of the excess fecal nitrogen (Mitjavila *et al.*, 1977).

We have been saying that tannin binds protein and therefore damages the nutritional status of the animal. This statement can be turned around to say that protein binds tannin, and to the extent that it is successful in carrying tannin away in the feces it prevents other serious toxic effects. In living plant material, tannins and proteins are naturally compartmentalized away from each other. During feeding and food preparation, the tannin and protein may contact each other. Heating of field beans, for example, decreases the toxic effect of the tannin, perhaps by enabling tannin-protein complexing to take place (Guillaume and Gomez, 1977). Intimate mixing of protein and tannin prior to their reaching more sensitive portions of the gut should diminish direct membrane effects, absorption, and systemic toxicity. In ruminants the extra mastication, saliva, and rumen fermentation should, therefore, be a safety factor.

The apparently greater toxic effect of tannin when administered by stomach tube in acute oral toxicity studies, or in drinking water (Patay *et al.*, 1962) or enemas (Boyd and Boyd, 1973), is believed to occur because "bare" mucosa are in direct contact with the tannin. Tannic acid, so applied, appears to cause a localized destruction of the epithelium of the rat's gastrointestinal tract (Weinberg *et al.*, 1966). Young rats appear to be more susceptible and less tolerant than older rats. Early effects of tannin's contact with intestinal mucosa include removal of the mucus covering, irritation, and edema. With continued exposure, ulceration and necrosis may follow. Ulcer-forming action by tannin appears to be countered by tannin's ability to inhibit gastric secretion and pepsin's action, and to promote healing of experimental ulcers (Satoru, 1976).

Tannin has long been believed to delay passage of food through the intestine and to stop diarrhea (Frey, 1908). Perfusion of the mouse intestine *in vivo* with tannic acid at 1 gm/liter markedly reduced absorption of glucose and methionine (Mitjavila *et al.*, 1970). The effect is considered a denaturation of the proteins of the membranous surface and presumably corresponds to the early-stage effects just mentioned.

This brings us to the question of whether tannin can be absorbed into the system through the intestinal wall. Different answers have been given at different times. Very early workers said that the tannin must be absorbed because it cannot be found in the feces. We now know that this is a false assumption, based on the difficulty of recovering tannin from its protein-bound condition in the feces. Another group said that tannin must be absorbed because phenolic products are excreted in the urine, but they were countered by a group that noted that the breakdown products may have been absorbed, and not the intact tannin. For a long time it was believed that, as precipitating macromolecules, tannins could

not be absorbed through normal proteinaceous animal membranes. Perhaps the key word is normal, but it does appear that tannin is difficult to absorb.

Korpásky and co-workers (1951) reviewed previous studies and presented experimental evidence that after tannic acid solutions were administered by stomach tube to rabbits and dogs "tannin" could be found in blood by colorimetry of the plasma. Three hr after a dose of a 2.5% tannic acid solution was administered, 20 mg/liter was found in plasma samples, and after 5% solution was given, 40-50 mg/liter was found. However, these workers recognized that their analytical procedure did not distinguish between gallic acid and tannic acid; in fact, administration of 2.5% gallic acid produced the same blood rise as did 5% tannic acid in their tests. Although they concluded that tannic acid can be absorbed intact, they did so on the basis that previous work by their group and others showed that gallic acid given parenterally or orally does not produce liver injury, whereas tannic acid does. This report has been cited (Boyd and Boyd, 1973) as demonstrating the direct absorption of tannic acid, but that is not entirely correct.

The most convincing direct evidence encountered to date is based on the absorption of a radioactive dimeric condensed tannin fraction from grapes. Within 10 min after oral administration of 2 μ Ci of such tannin, relatively small but appreciable amounts of radioactivity appeared in mouse blood (Laparra *et al.*, 1977). The blood radioactivity reached a maximum at 1 hr, and considerable excretion occurred via the bile. If the preparation was free of monomeric flavonoids and if no breakdown occurred during preparation or in the acid conditions of the stomach, this proves that absorption of intact dimeric catechin tannins took place. The appearance of radioactivity in the blood was much faster than the appearance of marked CO₂ in the breath or of phenolic acids in the urine, showing that extensive metabolic breakdown had not occurred (Laparra *et al.*, 1977). The radioactivity, as indicated by organ analysis and radioautography, concentrated in the liver, blood vessels, and bile duct tissues and in the mucous membranes rich in glycosamine glycans. This same pattern, however, was shown by monomeric taxifolin. Rutin was poorly absorbed in comparison tests (Laparra *et al.*, 1977). Histology of animals fed tannic acid at high levels shows effects that are different from those caused by gallic acid alone, and a rather specific type of coagulation necrosis seems common to liver, heart, kidney, and intestine cells (Karim *et al.*, 1978; Panda *et al.*, 1979). Again, failure to distinguish between tannic acid and the different natural polyphenolic mixtures of tea, coffee, and other foods has marred the studies.

It is too soon for an unequivocal answer as to whether large tannin molecules get through normal gastrointestinal tissues to cause systemic effects. Normally there are formidable barriers to this happening, and if any portion gets through it must be very small. It is clear, however, that free tannin in appreciable concen-

tration can damage the gastrointestinal surface. Exposure of burned skin surfaces or damaged colon tissue has produced fatal liver damage in humans. Incubation of tannic acid with intestinal fluid liberates gallic acid (Kerzanoski, 1966).

Although gallic acid at levels equivalent to those of tannic acid does not produce the same effects, since gallic acid is not a protein precipitant, we have yet to prove that the external effects of tannin plus the internal effects of released gallic acid, and perhaps galloylglucose, etc., do not account for the effects of oral tannic acid. Gallic acid at 5% in the diet produced fatty livers in rats, whereas 5% tannic acid did not (Mueller, 1942). In this regard at least, gallic acid was more toxic than tannic acid. Whereas in feeding trials on chicks the growth-inhibiting effect of gallic acid was only 30% that of tannic acid, methyl gallate was twice as active (Kratzer *et al.*, 1975). Alternative explanations for differences between the toxic effects of high-level tannins and their more obvious breakdown products seem possible. In chronic feeding trials, protein and other nutritional deficiencies caused by tannin, and not by catechin or gallic acid, should contribute to different toxic patterns. In acute studies, the extra gastritis, etc., could so contribute. Breakdown products, particularly of hydrolyzable tannins, may be poor protein precipitants, quite absorbable, and possibly more toxic than gallic acid.

The liver is subject to serious damage from tannin injection and, under some circumstances, from oral intake. The gross and ultrastructural changes in liver caused by injected tannic acid are reported to have much in common with changes caused by other hepatotoxins, such as carbon tetrachloride (Arhelger *et al.*, 1965; Drill, 1952). The central liver lobule is more involved, chronic doses give diffuse fibrosis, and massive doses cause necrosis. Oral administration of 50–300 gm of tannic acid to horses gave characteristic symptoms, including anemia, jaundice, and severe bilirubinemia (Begovic and Ozegovic, 1959), indicating overloading of the liver detoxication mechanisms. On the other hand, feeding rats 32 gm of tannic acid per kilogram of diet for six months did not affect the liver's functional status, triglyceride level, or oxidative enzyme level, although growth was retarded (Mitjavila *et al.*, 1971).

At cellular and biochemical levels, injection of tannic acid produces liver polyribosome disaggregation, affects microsomal enzymes, and inhibits nucleic acid and protein synthesis (Badawy *et al.*, 1969; Gaillard *et al.*, 1974; Oler *et al.*, 1976; Reddy *et al.*, 1970). Within 1 hr after intraperitoneal injection, tannic acid was found by solvent extraction and paper chromatography in liver cells, and by 3 hr it was concentrated in the nucleus fraction (Badawy *et al.*, 1969). The time sequence of events was concentration in the nuclei, inhibition of nuclear RNA synthesis, inhibition of protein synthesis, and, finally, production of necrosis. Cellular necrosis was well marked only after 24 hr following injection. Disaggregation of liver polysomal patterns and ultrastructural changes in the rough endoplasmic reticulum of rat liver cells began 3 hr after subcutaneous

injection of tannic acid at 700 mg/kg and were complete by 4–10 hr; recovery was evident by 72 hours (Reddy *et al.*, 1970). Tannic acid injected intraperitoneally at 25 mg/kg for 4 days produced a reduction in microsomal protein and certain induced detoxication enzymes, but not in others (Gaillard *et al.*, 1974). Concentration of cytochrome P-450 was not reduced, and pentobarbitone sleeping time was prolonged. Gallic acid, ethyl gallate, and a series of related acids at 150 mg/kg had no microsomal effect. Oral dosage of 2000 mg/kg of body weight to rats caused hepatocellular changes similar to those observed after 700 mg/kg given parenterally (Oler *et al.*, 1976). There was more inhibition of RNA synthesis in young rats than in mature rats. Tannic acid resulted in degranulation of rough endoplasmic reticulum, inhibition of protein synthesis, and disaggregation of polysomes. However, no evidence of single-strand breakage in DNA was detected. Degranulation of rat liver microsomes has been proposed as a quick test for carcinogenesis (Gupta and Dani, 1979). In this test tannic acid is active, but the connection between this finding and carcinogenesis seems tenuous and unconvincing.

Not just the liver is affected by tannic acid, as many studies have shown. Nonlethal injection produced proteinuria in rabbits in 24–96 hr (Boler *et al.*, 1966). Renal cell and mitochondrial alterations were confined almost exclusively to the proximal convoluted tubules. Effects were visible by 1 hr, and after 7 days the tissues had returned to normal. The feeding of 2–3% tannic acid to chicks produced “coagulation necrosis” in the liver, kidney, and intestine, as well as various other degenerative changes in these organs, desquamation of the intestinal epithelium, and hydropic degenerations of pancreas islet cells (Karim *et al.*, 1978).

Metabolism of tannic acid in animals produces gallic acid derivatives, mainly 4-methoxygallate. In chickens, after oral administration of tannic acid, gallic acid occurred in urine but not in feces, and pyrogallol was found in both (Kadirvel *et al.*, 1969; Potter and Fuller, 1968). The special ability of methionine and choline to alleviate tannic acid toxicity obviously relates to the availability of methyl groups to methoxylate gallic acid during detoxication. Pyrocatechol also was formed, presumably by decarboxylation and dehydroxylation. The decarboxylation products were produced whether or not the cecum had been removed (Kadirvel *et al.*, 1969). Metabolites of condensed tannins have been studied less. Carob tannin produced gallate metabolites in rat urine from the hydrolyzable tannins or epicatechin gallate present, but the catechin tannin portion did not undergo metabolism (Tamir *et al.*, 1972). With avocado and cacao preparations of mainly dimeric catechin tannins, intraperitoneal LD₅₀'s in mice were, respectively, 340 and 857 mg/kg (DeOliveira *et al.*, 1975). Intraperitoneal injection of either preparation at 80 mg/kg gave an analgesic effect (response of feet to heated surface) similar to that of morphine at 10 mg/kg and prolonged pentothal sleeping time. The animals were back to normal by 90 min. Human patients also reported

relief of pain after intravenous administration of these substances (DeOliveira *et al.*, 1975). Analgesia produced by narcotics in rats was substantially prolonged by subcutaneous administration of a zinc tannate combination (Brands *et al.*, 1980). Areca nut (betel) tannin (condensed) potentiated acetylcholine and nor-adrenaline action on rat tissue preparations *in vitro* (Sirsi *et al.*, 1966), and other reports suggest central nervous system reactions to tannins.

A condensed tannin of 2000–3000 molecular weight bound to pig myocardial membranes in the order sarcolemma > mitochondria > sarcoplasmic reticulum (DiPietro *et al.*, 1977b). The binding was enhanced by cations, but did not modify energy-linked calcium-ion accumulation in intact mitochondria, nor did it modify respiratory activity. The tannin behaved like a membrane stabilizer and prevented passive efflux of protein and cations. It helped maintain high ATP and creatine phosphate stores by inhibiting ATPase and creatine kinase, but it had no effect on ATP synthesis by oxidative phosphorylation in intact mitochondria (DiPietro *et al.*, 1977a). Inhibition of ATPase depended on access to the enzyme, since inhibition was produced in submitochondrial particles, but not in intact mitochondria. Tannic acid has also been shown to act only with specific segments of the outer surface of human erythrocyte membranes and to be unable to reach enzymes inside the cell (Herz and Kaplan, 1968).

Tannins have been reported to have effects on anemia and blood constituents. Roy and Mukherjee (1979) found that tannic acid and tannins from *Eugenia* black plum or green banana had somewhat different effects, but when fed at about 0.5 mg/kg of body weight per day they significantly increased retention of iron and its incorporation into heme in rats made anemic by administration of phenylhydrazine. In normal rats iron excretion was enhanced. At 2 mg/kg of tannin per day, absorption of iron was inhibited in both normal and anemic rats. Several mechanisms appear to be involved. The tannin may help keep the iron reduced to the ferrous ion form for ready uptake and metabolism. It may help recover iron in reusable form from metabolic breakdown products of heme. It may protect erythrocytes from lysis. Begovic and co-workers (1978b) found that introduction of 1 liter of 7–10% tannic acid solution into the rumen of fistulated goats caused within 2–3 days erythrocyte lysis, hemoglobinuria, and hematuria. Severe hyperchromic, megalocytic anemia occurred 1–2 wk after the administration of tannic acid. It could be cured by repeated injections of vitamin B₁₂. A number of other blood elements were also affected by the tannic acid. A diet of dry oak leaves produced some of the same effects, but not others, and the effects were considerably less severe.

Instances of the toxicity of tannin to farm animals have been observed for a long time. Periodic episodes of loss of stock from eating acorns have occurred in Europe (Clark and Cotchin, 1956; Wolter, 1974), notably in England in 1868, 1870, 1884, 1900, and 1933. This effect is attributed to the tannin content of the acorn. The acorns of European oak, *Quercus pedunculata*, contain about 6%

tannin; they are not willingly eaten by laboratory rats or mice. The tannins of *Quercus harvardi* are estimated to have caused annual losses of cattle worth millions of dollars in the American Southwest, owing to the fact that the leaves and twigs are eaten heavily at times when little else is available (Hulbert and Oehme, 1965; Keeler *et al.*, 1978; Singleton and Kratzer, 1969, 1973). In both cases, the oak tannin is hydrolyzable gallotannin. The tannin from shin oak, *Q. havardi*, reached an oral LD₅₀ after 5 days of dosage with 6.9 gm/kg/day in rabbits (Pigeon *et al.*, 1962). With comparable tests, 50% lethality was produced by commercial tannic acid at 3.4 gm/kg/day for 10 days, and symptoms were markedly similar (Dollahite *et al.*, 1962). At 0.5 gm/kg/day for 15 days given orally to rabbits, the oak tannin caused a doubling of liver β -glucuronidase activity and increased the blood concentration of urea, hemoglobin, erythrocytes, and serum transaminase (Camp *et al.*, 1967).

Tannin was widely used medically as a burn treatment (for coagulating the burned surface, protecting it, and minimizing exudation from it) from 1925 until 1942, at which time it was shown that tannic acid was the cause of liver necrosis in burn therapy. Owing to the lack of communication during World War II, tannic acid was still used on burns. Its effects in liver damage were later discovered separately in Central European countries. Experiments showed that tannic acid was absorbed into the system from the burned surface. Similar effects on the liver were produced in experimental animals by injection or treatment of burned or otherwise damaged, exuding surfaces. It was found that condensed tannins were not nearly as likely to cause liver necrosis as was tannic acid.

In 1946, it was found that incorporation of about 2.5 gm of tannic acid in a barium sulfate enema cleared the mucus from the colon wall, stimulated colon contraction, and promoted adhesion of barium to the lining for improved x-ray examinations. Commercial preparations incorporating tannic acid for this purpose were made available to American radiologists in 1962 and were applied to an estimated 600,000 patients per year. Beginning in 1963, liver necrosis and eventually at least eight fatalities were reported, which were traced to the tannic acid enema (Boyd and Boyd, 1973; Kerzanoski, 1966; Singleton and Kratzer, 1969, 1973). The products and their use were withdrawn in this country in 1964. It was shown that tannic acid is about twice as toxic when administered via the colon as when given by mouth, and if sufficient volume is given, the enema may reach the small bowel (Kerzanoski, 1966).

The tannic acid enema can produce extensive local inflammation and mucosal ulceration in the colon and the cecum, acute hemorrhagic hepatic necrosis, and hemorrhagic cystitis (Dukes, 1975). The hepatotoxicity is dose-dependent and is enhanced by repeated tannin enemas, prolonged retention, or increased concentration of the tannic acid. Patients with preexisting ulcerative colitis or other damage to the colon wall (those most likely to be x-rayed) and patients who are juvenile, debilitated, or aged are more susceptible to hepatotoxicity (Dukes,

1975; Janower *et al.*, 1967; Singleton and Kratzer, 1969, 1973). Patients with mild or no effects generally recovered without lingering sequelae, but a few cases of fulminating liver disease appeared to be initiated in children. Serious toxicity from tannic acid in enemas was evidently rare. With proper selection of patients, limiting the tannic acid content to 1%, and elimination of repeated colonic application, liver damage from tannic acid in barium enemas is unlikely to be dangerous (Dukes, 1975). A study of 80 patients in terms of several parameters of liver function for 12–72 hr posttreatment led to the conclusion that, properly managed, the procedure was both safe and useful (Kemp Harper *et al.*, 1973).

Tannins have been implicated in carcinogenesis. Experimental production of tumors in rats was reported by Korpásky and co-workers beginning in 1949 (Korpásky, 1961; Korpásky and Mosonyi, 1950). Young albino rats were given tannic acid subcutaneously every 5 days for prolonged periods. Of 28 treated rats, 23 survived for 100 days; 13 of the rats had hepatic tumors, and 15 had cirrhosis. The tumors were generally benign nodules of 2–8 mm. Only by par-enteral treatment was cirrhosis, hepatoma, or cholangioma produced. No local tumors were found at the injection site. Skin ulcers formed by repeated subcutaneous injection of 1.5–2.5% tannic acid at the same site healed rapidly and completely when the site was changed. Skin ulcers produced by burning the skin, which was then painted daily for 400 days with 5% tannic acid, showed no local change, nor were there cirrhotic or precirrhotic changes in the liver (Korpásky, 1961). Later reports confirmed the experimental results and suggested caution regarding heavy human consumption of beverages with high tannin content (Korpásky, 1959, 1961). It was concluded that tannic acid was weakly carcinogenic and was influenced by dietetic and endocrine factors. For example, a high-casein diet produced some protection against lethal and hepatotoxic action, even with injection of tannic acid. Males were more susceptible than females.

In comparisons of various tannins (Kirby, 1960), subcutaneous injection with 1 ml/wk for 12 wk of a concentration low enough so that little or no ulceration occurred at the injection site caused “sarcomas” at the injection site only for condensed tannins and, in addition, caused liver tumors with hydrolyzable tannins. Gallic acid and catechin were negative at the doses tested. Consumption of the tannins at 0.1–0.5% in drinking water for 3–6 mo gave no tumors. The results suggest that the condensed tannins are bound to body proteins at the injection site, whereas hydrolyzable tannin or fragments thereof are translocated to the liver.

After injection of tannic acid at 0.75 mg/kg intramuscularly into mice every second week for 18 mo, no differences were detected between the treated and the control animals (Bichel and Bach, 1968). According to the literature, none of the tannin-induced tumors were transplanted to other animals or were otherwise proved to be malignant. Later work (Oler *et al.*, 1976) verified the typical

cellular changes in rat liver reported by others (Korpássy, 1961) after single oral doses of 2000 mg/kg or 700 mg/kg administered parenterally. Although RNA and protein synthesis was inhibited and cellular changes included disruption of the endoplasmic reticulum and polysomes, no strand breaks in DNA were detected. The hepatotoxic effects were again demonstrated, but the lack of a DNA effect cast doubt on their carcinogenicity. The daily intake by a 70-kg man of 560 mg of food-grade tannic acid was considered well within safety limits under normal circumstances (Oler *et al.*, 1976). The unconditionally acceptable daily intake by humans of food-grade tannins as evaluated by an international expert committee of scientists is up to 0.6 mg/kg body weight for Peruvian tara tannic acid and up to 0.3 mg/kg for Turkish, Chinese, and Sicilian sumac tannic acids (Bigwood, 1973).

Epidemiological studies have attempted to evaluate whether tannins have a role in unusual incidences of human cancer. There have been reports correlating unusual consumption of plant material rich in condensed tannin with unusual frequency of cancer of the esophagus and mouth (Morton, 1970, 1972, 1973; Raghava and Baruah, 1958). These correlations appeared to be suggestive, particularly with betel chewing in parts of Asia, consumption of bird-proof sorghums in parts of Africa (particularly the Transkei), and tea (particularly herbal teas) in other specific societies. Unfortunately, such population-diet correlations, even if statistically evaluated as significant, do not of themselves prove any cause-and-effect relationship. The herbal teas are generally poorly known and quite likely to contain other potentially toxic substances, even if they also contain tannin. The appearance of tumors was accelerated, but the overall incidence was the same when benz(a)pyrene in a single dose was followed by application of tea or oak wood extract to mouse skin (Bogovski and Day, 1977). Tannic acid or gallic acid had no effect.

The relative per capita consumption of solid fuels, cigarettes, coffee, and tea all have positive or negative correlations with incidences of some form of human cancer (Stocks, 1970). Tea usage, the one with appreciable tannin, gave a negative correlation with incidence of stomach and uterine cancer, but a positive correlation with intestinal (except rectum), lung, and larynx cancer. The incidence of esophageal cancer in Japan correlates positively with the habitual consumption of rice cooked in tea rather than water; however, it is customary to eat this rice at a burning hot temperature, cooked softer and served with more liquid than ordinary rice is (Segi, 1975). In the most exhaustive study of esophageal cancer to date, it was noted that the incidence of this cancer varies geographically more than does that of any other alimentary tract cancer, often over short distances (Warwick and Harington, 1973). There are about 5.8 cases per 100,000 white males per year in the United States and, for the highest incidence known, 110.5 cases per 100,000 in Turmenistan. Environmental influence is indicated and occupations correlate, but heavy smoking, copious consumption of alcoholic

beverages, poor nutrition, and high use of emetics appear to be better correlated with the incidence of esophageal cancer than does diet. In fact, consumption of sorghum is not high in the Transkei (Warwick and Harington, 1973). The use of alkali in the form of wood ash or lime with the betel or tobacco chew also appears to be predisposing. It is contended that smoking, related tobacco usage, and other agents (including tannin, perhaps) act on the esophageal epithelium. After many sustained insults, it becomes susceptible, resulting in a high malignancy rate.

To summarize quickly, tannins (hydrolyzable tannins especially) can be seriously toxic when injected, when consumed in large amounts (more than 1% of the diet) for a considerable time, or when applied in concentrated solution to burned or otherwise damaged tissue. Sufficient application of tannin solutions (again generally more than 1%) to mucosa results in removal of mucus, epithelial edema, irritation, and tissue breakdown, which may in turn facilitate absorption of tannin, thus increasing its toxicity. The carcinogenicity of tannin appears questionable and is certainly weak (Miller and Miller, 1979; Singleton and Kratzer, 1969, 1973). It may involve repeated irritation and cellular damage, rather like continued mechanical irritation, instead of true DNA mutagenic carcinogenesis.

G. LIGNINS

Lignins are a heterogeneous group of large, insoluble, three-dimensionally linked polymers. The monomeric units are derived mainly from coniferyl alcohol with (depending on the plant) sinapyl or *p*-hydroxycinnamyl analogs. It adds bulk to the diet, leading to poor digestibility and unpalatable toughness. It is not particularly inhibitory to microorganisms; white-rot fungi preferentially utilize it in wood rotting. It is, however, sufficiently resistant that soil humus (largely lignin residues) can survive in the soil for upward of 2000 yr (Alexander, 1965).

Lignin is considered inert in the diet, except that by linkage to plant carbohydrates and perhaps encrustation coverage it lowers the digestibility of plant material. The lignin content is about 2% in immature forage, up to 15% in mature forages, and about 25% in woods such as spruce (Baker *et al.*, 1975; Pigden and Heaney, 1969). Much of our knowledge about lignin in nutrition relates to the utilization of wood, straw, and related residues for ruminant feed. Frequently a small increase in natural lignin content causes a considerable decrease in utilization of forage feed. Chemical removal of lignin with sodium chlorite increased cellulose digestion by rumen microorganisms from 66% to 91%, but simple addition of lignin to insoluble cellulose had no effect (Barton and Akin, 1977). High-lignin feeds can be improved in digestibility by very fine grinding, swelling and modification by steam or alkali, or delignification by white-rot fungi or other chemical treatment (Baker *et al.*, 1975; Pigden and Heaney, 1969).

Lignin itself has been considered indigestible and has been used as a reference

marker (for that reason) to compare digestive removal of other feed components, even in ruminants (Barton and Akin, 1977; Porter and Singleton, 1971). The actual digestibility of natural lignin in sheep was 1.2–10.2%, but when purified lignin was added to rabbit diets 41–47% was not recoverable in the feces (Lenz and Schürch, 1967). The lignin is modified by passage through the digestive tract of the rabbit or sheep, the modification occurring mainly prior to the intestine. The lignin fraction isolated from rabbit feces had less carbon and methoxyl content and more oxygen and nitrogen than the purified lignin did. In sheep feces the methoxyl content was 19.2% less than in hay and 33.5% less than in straw (Porter and Singleton, 1971).

Lignin or lignin fragments have never, to our knowledge, been reported to cause any serious toxic effects. Lignin degradation products as phenolic substances are somewhat inhibitory, and feeds prepared from wood and containing them can be improved by their removal (Britton, 1978). As a phenolic macromolecule, lignin solubilized by alkali has some tannin-like ability to bind and inhibit active enzymes and other proteins (Naess, 1977). This may be the mechanism by which lignin can cause lower nitrogen retention by the body from the diet, as with tannin (Lenz and Schürch, 1967). These effects are relatively mild, however, and lignin appears unimportant in terms of animal consumption except as an indicator of low quality in forage.

Lignin as a component of dietary fiber appears to be needed and beneficial in the human diet; its level is probably too low in the modern diet. Much interest has been aroused by the relatively high incidence of colon cancer and coronary disease in developed countries, compared with that in less developed areas. At least part of the difference is attributed to a diet low in residue, high in meat, and high in refined carbohydrate in relatively affluent societies, versus a diet higher in plant material and crude fiber in poorer societies (Huang *et al.*, 1978; Inglett and Falkehag, 1979; Story and Kritchevsky, 1979).

The mechanical bulk of the indigestible fibrous plant material contributes to feces bulk and to regularity of defecation. This in turn contributes to a shorter residence time in the bowel and thus may lower the exposure to dietary or microbially generated toxins and carcinogens in the gut. Lignin is a major component of dietary fiber, but its role and the roles of hemicelluloses, pectins, gums, and cellulose components are not yet clear. Carbohydrates and not lignin seem important to the water-holding capacity of the feces. Lignin—or certain lignin fractions—seems to be the most important fiber component in adsorbing bile steroid and dietary cholesterol, in nitrite binding and thus in lowering the formation of nitrosamine carcinogen, and in inhibiting enterohepatic recirculation (Inglett and Falkehag, 1979). Lignin may also be important for its antioxidant behavior and ion exchange or chelation effects. So far, the effects of lignin in the diet appear to be favorable or inert, rather than toxic. Lignin is frequently associated with plant phenols absorbable by animals. Many such phenols have a

hypercholesteremic action, producing additional excretion of bile. Combining this action with the ability of lignin to adsorb and fix into the feces both dietary cholesterol and bile steroids would enhance the effect. Favorable participation by dietary lignin in the epidemiology of coronary disease as well as colon cancer seems reasonable.

V. MECHANISMS OF TOXICITY BY PHENOLS

Earlier reviews emphasized unusual phenols having high toxicity (Singleton and Kratzer, 1969, 1973). The actual mechanisms of the toxicities, as far as they were understood, often did not seem to be unique to the unusually toxic phenol. Phenols with diverse structures appeared frequently to have toxic effects and symptoms in common. The low toxicity of common phenols was considered primarily the result of efficient barriers and detoxications in animals. The greater toxicity of an unusually toxic phenol often seemed to be the result of a structure that survived and penetrated the normal barrier mechanisms rather than a qualitatively unique toxic interaction with the animal's biochemistry. It was concluded that a number of the relatively common chemical and biochemical properties of phenols are involved in producing toxicity, and that common plant phenols have a toxic potential if natural defenses are weakened, overloaded, or evaded. It was also noted that nontoxic common phenols frequently augmented the effect of toxic phenols.

The present review has approached the subject from the other direction, studying the reported effects of the plant-derived phenols widespread in animal and human diets. The hypothesis that common plant phenols may exhibit physiological effects and toxicity under special circumstances by mechanisms similar to those found in notably toxic phenols seems to be validated and strengthened. However, the capacity of the animal body to protect itself against such happenings is impressive, and it generally appears to be quite capable of withstanding natural occurrences.

Let us consider in order the mechanisms or routes to toxicity that are likely to be involved with common natural phenols. Of course, any one toxic phenol may use several of these mechanisms simultaneously or at different times. Discussion will be limited to what is deemed necessary to clarify without repeating previous comments. Under certain circumstances a potential for toxicity can confer a protective effect. Some of these will be mentioned.

Consideration of the structural details that make phenols capable of avoiding detoxication, and therefore presumably more toxic, easily becomes a circular argument. If the common phenols are nontoxic because they have been evolutionarily common, a mutation giving an uncommon structure has a greater chance of being toxic. On the other hand, we generally have too little information

concerning the effect of intermediate structural modifications to speculate very helpfully on structure-toxicity relationships. The most noted toxic phenols—gossypol and podophyllotoxin, for example—tend to be complex and quite different from common phenols. Some toxic phenolic derivatives—coumarin and safrole, for example—are fairly simple and more similar to common phenols, such as *p*-coumaric acid and coniferyl alcohol, that are apparently nontoxic. The balance of water-lipid solubility (penetration, membrane interaction?) and protection from normal detoxicative metabolism and conjugation seem important (methylenedioxy substitution, no hydroxyls for sulfate or glucuronoside conjugation, etc.). Here we are focusing on the usual structures, so details of the unusual toxic phenols are left to other reviews (Singleton and Kratzer, 1969, 1973). To illustrate the universality of the various mechanisms, however, uncommon and toxic phenols will be mentioned.

A. MIMIC-INTERACTION WITH NORMAL PHENOL METABOLISM

The most often cited effect of phenols that falls under this heading is the inhibition of catechol-*O*-methyltransferase (COMT) and competition for methyl groups by phenols that serve as a substrate for this enzyme. As was already noted, certain flavonoids appear to inhibit the enzyme without serving as a substrate and being methoxylated (Kühnau, 1976). Many catechol derivatives apparently do compete as substrates, deplete the available methyl group supply, and inhibit normal action of the COMT enzyme (Baraboi, 1967; Dorris and Dill, 1977). The adrenergic fibers of the sympathetic division of the autonomic nervous system produce action in the innervated organ by liberation of norepinephrine and epinephrine, catecholamines produced from 3,4-dihydroxyphenylalanine (dopa) (Daly, 1967). The stimulus is terminated by methylation of the epinephrines to inactive metanephrines. If the xenobiotic phenol reaches the nerve junction and inhibits or competes for the COMT action, it will have a sympathomimetic effect, prolonging the actions of the epinephrines. If it acts only by seriously depleting the active methyl group supply via liver COMT, it also can prolong the action of catecholamine; but if it inhibits COMT consumption of methyl groups at the liver, it may free activated methyl groups for inactivation of catecholamines at the nerve (Chaudhari and Hatwalne, 1977).

Choline in the form of acetylcholine is the effector-initiator in the cholinergic fibers of the parasympathetic division of the autonomic nervous system. Choline, a source for active methyl groups besides methionine, is subject to dietary inadequacy and depletion if there is a high demand for methyl groups. Furthermore, effects on the central nervous system are frequently noted with administration of phenols (Brewster *et al.*, 1977), which might result from competition of these or other types possibly involving dopa and its metabolites. Hydroquinone inhibits melanization reactions involving tyrosinase and dopa, and its use results in

depigmentation of hair and skin of black animals. Nerve transmission might be affected by $\text{Na}^+ - \text{K}^+$ ATPase inhibition, which has been reported for some phenols (Mirsalikhova and Pakudina, 1977). Salicylate analgesia apparently has some weak corollaries with more common phenols (DeOliveira *et al.*, 1975), and the anti-inflammatory activity is widely shared.

Sympathomimetics commonly induce tremor in animals. Tremor, convulsions, and other nerve-related symptoms are very common in poisoning with natural phenolic intoxicants such as tremetole, hypericin, marijuana, mescaline, nutmeg, kava, and safrole (Singleton and Kratzer, 1969, 1973). The related effects with common phenols occur less frequently and are much milder, yet the common phenols should be as great or greater consumers of methyl groups in terms of the metabolic load. This seems to suggest that the involvement of the common phenols with COMT is effectively handled at the liver, and nerve involvement is minimized.

The competitive consumption of iodine by common phenols results in hypertrophy of the thyroid (goiter) in the body's effort to produce thyroxine (Moudgal *et al.*, 1958; Jeney, 1968). This very weak goitrogenic activity probably requires borderline iodine deficiency and high phenol intake to be significant. Such conditions can, however, occur naturally in humans, even today. This is one reason for the common observation that toxic effects are magnified by unsatisfactory diet or poor health.

Other mimic-interference toxic effects of certain natural phenols—for example, the estrogenic action of certain isoflavones, coumarins, and phenolic mold products and the antivitamin K effect of dicoumarol—do not appear to be significant for any of the common dietary plant phenols.

Protective effects are observed when detoxication of common natural phenols stimulates the production of the microsomal mixed-function oxidase systems and probably the conjugation systems. The induced enzymes are frequently capable of metabolizing other substances and reducing their toxic effect at a given initial dose. This could fairly be considered a mimic-interaction situation, examples of which were cited earlier (Cutroneo *et al.*, 1972; Wattenburg *et al.*, 1968). The role of the common plant phenols is probably more important, since advance exposure is regular, but some special cases that may involve this effect are actively under study. An example is silymarin protection against amanita toxin, although membrane stabilization appears also to be involved (Antweiler, 1977; Faulstich *et al.*, 1980).

B. NUTRITIONAL LOAD

The debilitating drag of the necessity to inactivate or detoxicate a sizable intake is probably the most general naturally significant "toxic" role of the common plant phenols. It is usually somewhat less important for the acutely

toxic but rarer phenols, since it is likely to be manifest only in chronic conditions of marginal or deficient nutrition. The requirement for extra methionine or another methyl-group source when phenols are consumed has just been mentioned, but it is relevant here as well. Dietary supplementation with methionine or choline has often removed a major portion of the negative effects of a high-phenol diet, as was mentioned in the discussion of the effects of gallic acid and tannic acid. A lack of methyl-group donors in the diet produces in poultry a characteristic deficiency disease syndrome called perosis, involving hock joint abnormalities that prevent normal walking. Although the relationship is not always recognized, the symptoms seem essentially the same as those produced by high tannin intake, and they can be at least partly overcome by intake of methionine (Breider, 1968; Elkin *et al.*, 1978a,b; Rungruangsak *et al.*, 1977).

Supplementation with other amino acids may be helpful in dietary toxicity of phenols, but the effects generally appear to be nonspecific and only alleviate poor nitrogen nutrition. Effects of tannins that make dietary protein unavailable by "tanning" it, that inhibit and cause extra production of digestive enzymes, that cause losses requiring replacement of the mucus lining of the alimentary tract, or that throw animals consuming large amounts into negative nitrogen balance have been discussed. Similar, albeit much smaller, effects appear to be produced by other phenols in proportion to their ability to bind protein.

Small phenols as well as larger ones also cause nutritional drag by consuming energy in detoxication reactions (methylation, sulfation, glucuronide formation, etc.) and by the loss of the substance of the methyls or glucuronic acid from more useful metabolism. Stimulation of the flow of bile and urine to excrete the phenolic detoxication products has a potential health value in the removal of cholesterol and other waste products, but in a starvation situation it may also contribute to depletion.

The magnitude of common phenol intake necessary to produce a significant nutritional load effect appears to be in the range of 1–5% of the diet, since that is the range that generally causes the first long-term toxic effect of feeding bioflavonoids, tannin, gallic acid, tyrosine, and other common natural phenols. The range is higher (that is, a higher percentage is required to produce toxicity) for insoluble phenols like ellagic acid and lignin, and lower for more specifically toxic phenols. This shows that the normal protection and detoxication mechanisms have a rather large capacity.

Ordinarily, innocuous common phenols compete with more toxic phenols and other toxins for much the same maximal capacity of microsomal mixed-function oxidases and other detoxication mechanisms. This situation explains the observation that the isolated active principle is often not as toxic in unadapted animals as is the whole crude source. For example, nutmeg volatiles contain the toxic phenol derivative myristicin, and the nonvolatile phenols are not toxic (Singleton and Kratzer, 1969). However, feeding the two fractions together increases the toxic-

ity of the myristicin. Synergy—an increased toxic effect caused by the addition of normally nontoxic phenols with a borderline dose of a toxic phenol—is usual. This effect may be obscured under chronic administration if the induction of detoxication enzymes enables them to cope with the total load. If the maximum inducible detoxication is exceeded in normal animals or is lowered by poor health, the common plant phenols will contribute to the overall toxic effect.

The diet may be made inadequate in other ways by the presence of phenols. Caffeic acid (Schaller *et al.*, 1977; Somogyi, 1978; Somogyi and Nageli, 1976; Swanowska and Tautt, 1972), tea flavonoids (Rungruangsak *et al.*, 1977; Kositawattanakul *et al.*, 1977), and perhaps other common phenols can have antithiamine effects. Adducts are formed that make the thiamine unavailable. Oxidation is involved, and ascorbic acid prevents this antithiamine effect. Tannic acid precipitates vitamin B₁₂, and the precipitate passes through the rat, making the B₁₂ unavailable and contributing to anemia (Carrera *et al.*, 1973). This is thought to be one reason for the common anemia found among strict vegetarians. Another factor can be chelation, which renders the iron of the diet unavailable. Supplementation of the diet with extra iron helps counteract the toxic effects of gossypol, tannin, and some other phenols (Singleton and Kratzer, 1969, 1973). The chelation of metals by properly substituted natural phenols can be also helpful. Mice with 2% tannic acid and 3% lead acetate in their diet survived for 13 wk; those receiving only 3% lead acetate all died before 13 wk (Peaslee and Einhellig, 1977).

C. PENETRATION EFFECTS

Most biological membranes at the cellular level have a lipoprotein or related biphasic nature so that substances readily interacting with the membrane need some water solubility, but also some lipid solubility and limited polarity. Glycosides of phenols are generally less toxic when given orally and often when given parenterally. The glycosides are not readily hydrolyzed by the animal unless by the gut flora, and they are not readily absorbed until hydrolyzed to the aglycones. If injected, the phenolic glycoside, like the conjugate glucuronoside, is prone to rapid excretion.

Aglycones of flavonoids are readily absorbed, particularly the ones like the catechins, which usually occur free and not as glycosides in plants. Flavonoids and smaller phenolic derivatives, which lack free phenolic groups, are not subject to sulfate or glucuronoside conjugation. These tend to be rarer in nature and more toxic. Urushiol, the toxic allergen of poison ivy and its relatives, penetrates the skin quickly and binds there (Singleton and Kratzer, 1969, 1973). Phenol itself is a protein solvent and facile skin penetrant with appreciable lipid solubility. Feeding myristicin and several other phenolic toxins with lipid or as fine

dispersions—for example, kava constituents—increases their toxic effect, presumably by increasing their solubility and absorption via chylomicrons with the fat. The toxicity of safrole, coumarin, psoralen, and rotenone may depend partly on their lack of free hydroxyls and their high lipoidal membrane solubility. The collagen of capillary vessel walls is more retentive in the presence of bioflavonoids. Small phenols, like other penetrants, may carry solutes through tissue barriers.

Completely methoxylated flavonoids like tangeretin and nobiletin also have high lipid solubility and cannot be conjugated in the usual way. Additional methoxyls on the A-ring presumably make this structure difficult to break down metabolically, when compared with the normal phloroglucinol ring. Yet the high methoxyl content would confer some hydrophilic character. These and related compounds are rather restricted in their botanical occurrence and appear to have unusual but rather weak antifungal and cytotoxic effects, as was already noted.

D. PROTEIN BINDING

Although tannins are protein binders without peer, the same hydrogen-bonding mechanism is available to smaller phenols and appears to be important in some of their physiological effects and toxicity. Salicylates bind to blood proteins (Singleton and Kratzer, 1969). Injected tannins must also combine with blood proteins. Gossypol accumulates in the body by attachment to lysine of body (especially liver) proteins via a carbonylamine Schiff's base—not because of its phenolic functions, but its action illustrates the process nevertheless (Singleton and Kratzer, 1969, 1973). The hydrogen bond from phenol to protein is stronger, the more acidic the phenol (Wade *et al.*, 1969). The pK_a of simple phenols correlates well with their uncoupling effect on oxidative phosphorylation and binding with serum albumin.

Although their role is generally reported favorably as a stabilizing effect, catechin and some other common plant phenols have demonstrated *in vitro*, and sometimes *in vivo*, activities that bind them with collagen (Comèl and Laszt, 1972), other proteins, enzymes (Niebes, 1973), and membranes (Farkas *et al.*, 1977; Ring *et al.*, 1976). Tangeretin (Robbins, 1977; Robbins *et al.*, 1971) and ellagic acid (Botti *et al.*, 1965; Girolami *et al.*, 1977) have effects on blood cell aggregation and clotting that presumably involves bonding with proteins or cell membranes. The effects of tannin on dietary protein are similar. The antimicrobial and antiviral activities of tannins and often of other phenols appear to involve adsorption and binding to the microbe or virus surface, thereby inhibiting infectivity and growth. The enzyme-inhibiting and mucus-removal effects of tannins and related substances involve tanning-like reactions with enzyme protein, mucoprotein, or glucosamine glycans.

E. RESPIRATION EFFECTS, UNCOUPLING OF OXIDATIVE PHOSPHORYLATION

Phenols are noted for their uncoupling of oxidative phosphorylation and related effects. Rotenone is widely used in enzyme biochemistry as a specific inhibitor of NAD to coenzyme Q oxidation early in the electron transport respiration series. Synthetic phenols, notably 2,4-dinitrophenol, are active uncouplers of oxidative formation of ATP. Dicoumarol has uncoupling activity and many other natural phenols do also, to a much smaller degree. The phenols with the most activity as uncouplers of oxidative phosphorylation are generally also those that have the greatest acidity, are more lipophilic, and are relatively more toxic, at least among fairly simple phenols (Fujita and Nakajima, 1969; Tollenaere *et al.*, 1976; Wada *et al.*, 1969). The more oxidizable phenols are likely to be more nephrotoxic (Calder *et al.*, 1975).

The tendency of vegetable phenols to stimulate the basal metabolic rate (McLaren *et al.*, 1973; Stelig and Qasim, 1973) appears to be related to the effect of phenols on respiration metabolism (Gadjos *et al.*, 1969; Graziani, 1977; Mal'yan and Akula, 1977; Suolinna *et al.*, 1974, 1975). The effects of phenols on ATPases also may play a role (Fewtrell and Gomperts, 1977; Mirsalikhova and Pakudina, 1977). It seems significant that the essential phenols—vitamins E and K—are involved in oxidation-reduction metabolism. Phenols of common types might affect poisoning, autoxidation, and oxidation-reduction reactions. Some less common but natural phenols and their quinones (for example, hydroquinone, juglone, anthraquinones, and pyrogallol) certainly appear to owe some of their toxic properties and sometimes (as with antioxidants) their beneficial roles to these effects. The bioflavonoid relationship with vitamin C is at least partly the protection of minimal amounts of ascorbic acid from oxidation.

The rather specific effects of common flavonoids on cAMP phosphodiesterase, and on some active transport enzymes discussed earlier, have not been noted to be part of the mechanism of any uncommon toxic phenol. They may be part of the normal control mechanisms in plants.

F. MUTAGENICITY, CARCINOGENICITY

Aflatoxin is a uniquely carcinogenic phenolic toxin. Its carcinogenicity appears to involve activated furan rings, and its structure is quite unlike that of any common plant phenols. Safrole is evidently a rather weak esophageal carcinogen with an unusual methylenedioxy grouping (Singleton and Kratzer, 1969, 1973). Carcinogenicity has not often been a demonstrated feature of phenolic toxicity, but some of the most potent carcinogens—aflatoxin, for example—are microbial phenols (Miller and Miller, 1979).

The putative carcinogenicity of tannins appears to be very weak, if real. It

seems to be related more to severe and prolonged irritation than to true carcinogenicity. The necessary experimental conditions are very unlikely to occur naturally. The most thorough epidemiology (Warwick and Harington, 1973) and evidence for lack of DNA reactivity cast doubt on any carcinogenic danger from tannins (Korpáßy, 1961).

The recent indications of mutagenicity for quercetin and related flavonols in the Ames-type *Salmonella* mutant tests are somewhat balanced by the associated findings that the structural requirements for mutagenicity exonerate most flavonoids (Macgregor and Jurd, 1978). The effect of quercetin was one to three orders of magnitude less than that of known powerful carcinogens, although apparently it was stronger than that of dimethylnitrosamine or benzidine (Sugimura and Nagao, 1979). In view of a considerable experimental and clinical history with quercetin derivatives without evidence of cancer initiation, the risk must be small. Quercetin is commonly stated to be the most widespread of flavonoids if not of phenols in higher plants. Of course, the risk to animals and humans cannot yet be considered nil; more study is clearly required.

Naphthols, nitrophenol, and chlorophenols produce "stickiness" and abnormalities in plant chromosome behavior during meiosis (Amer and Ali, 1968), but common plant phenols apparently do not. Damage to DNA as indicated by strand separation was caused by rotenone and 2,4-dinitrophenol, but upon removal of the agent the DNA was repaired (Hilton and Walker, 1977). Perhaps interference with the supply of ATP and the energy requirement for DNA repair was involved. The furanocoumarin, psoralen, intercalates DNA and upon irradiation covalently links to it (Singleton and Kratzer, 1969).

The role of phenols as cocarcinogens or as a cause of tumors after an initiating contact with a known carcinogen is apparently variable. In experimental applications of cigarette smoke condensate, catechol was an active cocarcinogen, but phenol was an inhibitor (Van Duuren *et al.*, 1973). The chlorogenic acid content of coffee is considered a cocarcinogen in the sense of catalyzing the formation of *N*-nitrosamine carcinogens from nitrate and secondary amines (Challis and Bartlett, 1975; Mori and Hirono, 1977). In other studies, however, gallic acid and tannic acid were found to be effective inhibitors of nitrosation at gastric pH (Mirvish *et al.*, 1975).

The embryotoxic effects reported for tangeretin may be at least partly due to the undeveloped detoxication mechanisms of the embryo. Of course, cytotoxicity as demonstrated in cell culture cannot involve the liver metabolism etc. of the intact animal. Synthetic phenylethylphenols applied to mouse skin at 25% in olive oil produced no visible effect on the adults, but decreased the frequency of pregnancy and the size of the litter (Broitman *et al.*, 1966). The treatment had more effect on males and was said to show the same trends for three generations after the treatment! In guinea pigs, teratogenic as well as mutagenic effects were observed.

It is interesting that antioxidants like butylated hydroxyanisole are potent and wide-spectrum inhibitors of carcinogenesis (Wattenberg, 1980). Quercetin and many other common natural phenols are rather good antioxidants. Induction of increased microsomal mono-oxygenase activity by plant phenols could enhance carcinogenicity, if the carcinogen requires metabolic activation, but more commonly it inhibits carcinogenesis. Penta-*O*-methylquercetin inhibited experimental pulmonary adenoma induction by benz(*a*)pyrene (Wattenberg, 1980).

G. MISCELLANEOUS EFFECTS

Other properties of phenols have been mentioned in connection with phenol toxicity and physiological activity. Chelation of iron and other metal ions by phenols, common or otherwise, can be involved in development of anemia, removal of lead toxicity, etc. Keeping iron ions reduced to the more useful ferrous form can be a role of readily oxidized phenols. The specific inhibition of histamine-forming enzymes has been reported for catechin, and various phenols have been reported to cause release of histamine from mast cells, although others do not.

Inhibition of prostaglandin synthesis has been reported for some natural phenols; it may be related to spasmolytic or possibly to toxic effects. Aspirin and polyphloretin phosphate have been particularly active prostaglandin antagonists (Sanner and Eakins, 1976).

Phenols are not very allergenic, with the exception of poison ivy urushiol and its relatives. Sensitivity reactions have been reported with vitamin E aerosols and *p*-hydroxybenzoate ointments. The coffee allergic reactions attributed to chlorogenic acid seem to be caused by contamination with coffee protein. Histamine-release responses to phenol administration are variable and usually weak. Catechin appears specifically to inhibit the enzyme that produces histamine from histidine in the stomach.

VI. CONCLUSIONS AND ASSESSMENT OF RISKS

The reported incidences of toxicity or appreciable physiological effects in animals caused by common plant phenols have been reviewed and interpreted. A number of conclusions and tentative conclusions may be drawn.

Many plants contain secondary metabolites toxic or deterrent to members of the animal kingdom attempting to feed on them. Phenols are an important group of these secondary plant substances; they may be divided into groups according to their common, uncommon, or rare occurrence in nature. Plant phenols that are highly toxic to animals are rarely found. The phenols that occur widely in plants

or in large amounts in common food plants have very low acute oral toxicity, and most are also of low toxicity when given parenterally.

A case has been made, however, that the mechanisms of toxicity of plant phenols in animals are frequently based upon characteristics shared generally by phenols of both high and low toxicity. The difference is attributed in part to efficient detoxication in the animal of the common phenols. Toxic phenols are unsatisfactorily metabolized because of structural features (or inappropriate, abnormal administration) not readily withstood by the normal animal barriers. The toxicity of various types of plant phenols appears to be an important aspect of their evolutionary advantage to plants; minimizing feeding by herbivores certainly can be one advantage.

Animals, particularly mammals, have evolved to tolerate ubiquitous secondary plant products, including most common plant phenols. The major detoxicating mechanisms not only improve with challenge during evolutionary contact, but they are often quickly adaptable and may be induced in the exposed individual to cope with extraordinary types and amounts of would-be toxicants. Herbivores, especially ruminants, through a combination of alimentary tract microbes and evolutionary adaptation of their detoxication capacity, tolerate a considerable intake of common plant phenols without evidence of toxic symptoms.

Carnivores, and other creatures not adapted to plant diets, appear to be deficient in detoxication capabilities. Man, as far as the data indicate, seems to be rather tolerant to plant phenol toxicity, compared with most experimental animals. Experiments with primates other than man support this conclusion. The human fetus and newborn seem to have a greater and earlier detoxication capacity than do those of laboratory rodents and other experimental animals. Detoxication capacity in the adult is greater in total, is larger in proportion to body weight, and is more likely to have been already induced by previous exposure to the same or similar compounds. One of the advantages of the normal human on an eclectic diet is that such a diverse diet should give regular exposure, adaptation, and relative detoxication tolerance toward all common plant constituents without excessive consumption of any one food source or constituent.

Smaller phenols may be a practical risk factor as cocarcinogens, especially in smoking, but even in smoked foods they are present in such small amounts as to appear safe in terms of direct effects from dietary consumption. The common hydroxycinnamates, hydroxybenzoates, and flavonoids are relatively innocuous, even with elevated and prolonged consumption. Most reports, in fact, suggest favorable effects or even "semiessential" roles (Kühnau, 1976). These physiological effects are generally mild, and the low toxicity has led to considerable experimentation and clinical application, particularly of the so-called bioflavonoids. Some iatrogenic deaths and serious negative effects have been produced by rather heroic parenteral administrations—of poorly soluble quercetin

derivatives, for example, causing fatal liver concretion in infants. The risks from these compounds in normal food consumption appear to be nonexistent, with the possible exception of the worrisome mutagenicity of quercetin and a few close relatives in mutant bacteria reversion tests and the experimental toxicity of completely methoxylated flavonoids, which are rare except in citrus peel oil. In both cases, the data suggesting a toxic risk are far removed from human experience. Further testing is required for a more certain conclusion. On both experimental and epidemiological grounds the risks so far appear to be very small.

Tannins are sufficiently concentrated in some plants and of sufficiently astringent and bitter taste that they are a deterrent to feeding by animals. Under normal ad lib consumption, if low-tannin feed is available, high-tannin feed is avoided by animals. Tannins quickly combine with proteins in the diet, and with digestive enzymes, mucus secretions, and microorganism or virus coats. This may lead to gripping diarrhea or constipation, reinforcing food avoidance reactions in the presence of tannins. The tannins are seen as plant protectants having this sort of "toxic" effect. The risk to humans or unconfined animals having a choice of diets seems very small. It is not small, however, when high-tannin food is the only choice, as has been proved by fatalities in animals. Human deaths as well as experimental toxicity have occurred from application of hydrolyzable gallotannic acid in concentrations of about 1% to burns, from enemas, or from smaller amounts given by injection. Condensed tannins are considerably safer, particularly with regard to liver damage.

The barriers that protect against liver damage, possibly including carcinoma, seem formidable, and the direct risk from dietary consumption seems very small, under all but the most severely restricted and high tannic acid conditions. Evidence of tannin involvement in other examples of "naturally" occurring toxic effects, notably esophageal cancer, has been reviewed and considered to be unconvincing.

The most practical risk to humans and other animals from high intake of common phenols appears to be from the extra load placed on the body in conditions of inadequate nutrition. Weakened, debilitated, infantile, senile, or traumatized animals on a diet inadequate or barely adequate, and without appreciable plant phenols, will find it even more inadequate with them (unless the most significant shortage is vitamin C). Depending upon the conditions, there are several reasons for this, which have been discussed.

When these findings are taken together with some considerations of the uncommon and highly toxic phenols, a fairly coherent picture appears. Common plant phenols are not invariably innocuous. But, when the known conditions necessary to produce deleterious effects are compared with those normally present, potential toxicity problems are unlikely to occur, are easily foreseen, and may be readily avoided.

VII. RESEARCH NEEDS

The crucial need, as this article is written, is to know whether or not the demonstrated bacterial mutagenicity of quercetin and a few related flavonols is of practical significance in human and animal carcinogenesis. Tests bearing on this question are known to be in progress with experimental animals. The intense or long-term animal feeding trials so far completed did not indicate appreciable toxicity, but they were not specifically designed to detect carcinogenesis. In view of the widespread occurrence but low content of quercetin in foods from plants, it is important to consider the problem carefully with input from toxicology, food science, statistics, and other disciplines. Shunning of all foods with traces of flavonols would be impractical and would eliminate most natural plant foods. A diet exclusively of refined carbohydrates and animal products is already known to be epidemiologically associated with more risks than a diet with more vegetable matter. Facts and studious evaluation must supplant piecemeal sensational reporting, if the public is to be best served.

The majority of the phenols common in foods have very low practical toxicity and are negative in the Ames test, and many have definite or possible dietary or therapeutic benefits. We need to know more about their fate in man. Testing both on physiological effects and on metabolism after oral intake appears safe and should be expanded. Further efforts to distinguish human metabolism from the metabolism of the gut microbes are needed. The careful investigation of useful dietary and therapeutic applications of the natural phenols and their derivatives has been unduly restricted in this country by the excessively negative evaluation of earlier "bioflavonoid" research. It is also clear that medical use in Europe has been occasionally excessive. More and better research is needed.

Expanded roles for anthocyanins to replace synthetic food colorants, for dietary fiber including lignin, and for vegetable versus animal foods indicate current trends. Continued research on the contribution of natural phenols in foods is warranted. New dietary compositions and food formulations suggest further research on plant phenols in relation to food quality and healthfulness. The antioxidant, metal sequestering, preservative, and other effects of natural plant phenols, when better understood, may enable them to replace synthetic additives and help further to maintain the healthful quality of the modern diet.

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