Toxicity and Related Physiological Activity of Phenolic Substances of Plant Origin

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The toxicity to animals of plant phenols is reviewed; particularly, hydroquinone, salicylic acid, coumarin, bicoumarol safrole, myristicin, urushiol, phloridzin, tangeretin, hypericin, psoralen, gossypol, rhein, sennoside, tetrahydrocannabinol, tremetone, dihydromethysticin, podophyllotoxin, rotenone, and tannin. General correlations and evolutionary significance are discussed. Phenols appear generally toxic if natural barriers or detoxification mechanisms are overloaded by amount, circumvented by the manner of administration, or foiled by uncommon compounds such as methylene diethers or isoprenoid structures. Frequently manifested features of phenol toxicity include synergism, bonding with body polymers, interference with metabolism of normal phenols (catecholamines, tyrosine, vitamin K), and involvement of the skin and liver.

It is rather difficult, perhaps impossible at this stage of our knowledge, to pull together into a satisfactorily coherent whole a subject as diverse and impinging upon as many disciplines as that indicated by the title of this report. It seems desirable, however, to avoid as far as possible the mere tabulation of a series of curiously toxic plant phenols. "Phenol chemistry" in living systems has not been as clearly organized as, for example, carbohydrate chemistry. Greater unification in understanding of the chemistry of natural phenols is finally emerging and perhaps toxicity and related physiological activity can serve as one key to furthering this trend.

The authors discuss the subject from several different aspects and, since complete literature coverage is impossible in the space available, cite primarily the most authoritative, provocative, or recent pertinent reports. The plant phenols to be considered do not include nitrogen-containing substances or triterperoids such as saponins or steroids, since the authors wish to focus on the compounds which owe their natural presence and perhaps their physiological effects to their phenolic nature. For convenience, the phenolic products of microorganisms and other lower plants have been excluded. On the other hand, substances are included which can be considered as derived from phenols such as chromones or coumarins whether or not they have additional free hydroxyls. Since toxicity is often manifested when "unusual" plant products are contacted by man or animals through ignorance or restricted access to normal food, discussion has not been limited to phenols likely to be present in foods or feeds.

Animals make very little direct use of the many phenolic substances of plants, with some outstanding exceptions such as tyrosine, the vitamin E tocopherols, the vitamin K naphthoquinones, and the ubiquinone benzoquinones. The physiological activity of these substances in a vitamin or desirable nutrient sense is outside our concern here. For the same reason discussion of the bioflavonoids (De-Eds, 1968) has been sharply limited.

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Phenolic substances are produced in animals for a few key functions, notably the catechol amines and phenolic indole amines involved in nerve action and associated effects, the tyrosine-dopa derivatives involved in melanin pigment formation, the phenolic steroidal estrogens, and the tyrosine of proteins. While the production of these compounds ultimately depends upon transformation of a benzenoid plant product, animals do not normally encounter these substances in ways which will disrupt their metabolism. It would seem evolutionarily essential that substances as potent in the bloodstream as norepinephrine or serotonin would not be readily taken up in active form from food plants which can contain them. Considering that one nettle sting may contain 10 times the 5-hydroxytryptamine of a bee sting and that 1 gram of banana may equal, in this particular regard, 50,000 bee stings (Ramwell *et al.*, 1964), it is fortunate that few plants are adapted for parenteral administration of toxins.

It would be reasonable to seek interference by "plant" phenols in the normal functions of phenols used by animals as a source of toxicity. However, potent analogs or inhibitors of essential "animal" phenols must be rare in common food plants, or animals must have effective "detoxification" mechanisms for common plant phenols. In the sense that there is no necessity to metabolize many plant phenols at all except to render them less toxic or hasten their excretion, detoxification is probably the proper term. However, as long as the detoxification system is efficient and not overloaded, toxic symptoms may never appear after ingestion of a potentially toxic phenol. Since many plant phenols are excreted partly unchanged, complete "detoxification" seems often unnecessary.

In contrast to the few phenols common in animals, plants produce many different phenols, vary them considerably plant to plant, and may contain them in small or very large amounts. A file based originally upon tabulations by Geissman and Hinreiner (1952) and by Karrer (1958) now lists about 800 different phenolic substances which fall in the group being discussed, not counting simple glycosidic variation, and is certainly not complete. If one selects from this group the phenols which are most

frequently present in plants as shown by chemotaxonomic examination (Bate-Smith, 1965, 1968; Bate-Smith and Swain, 1965; Swain, 1965), a familiar list of about 25 phenolics is almost universal in animal diets derived from plants. These include *p*-coumaric, caffeic, ferulic, sinapic, and gallic acids, and the common flavonoids with analogous variations of 5, 7, 3′, 4′, 5′-hydroxyl or 3′, 5′-methoxyl substitutions in the anthocyanidin, catechin, leucoanthocyanidin, flavonol, and flavone series. Ellagic acid and a few depsides and other derivatives would round out the list.

If, on the other hand, a list is prepared of the phenolic substances which have significant medical or toxic effects in animals and they fit our other restrictions (Stecher, 1968), about 150 are found. There is little overlap between the lists. None occurs if one eliminates the substances with reputed favorable effects such as the bioflavonoids and the nonspecific bitters and astringents and retains only those few dozen phenol-plant combinations which seem most significant from a toxicity viewpoint. Of course, a large portion of the total number of phenols reported from plants appear on neither the common nor the toxic list. Many of these have not been investigated from a toxicity viewpoint. A major factor shaping the list of the plant-phenol combinations with toxicity considered significant is a fortuitous combination of circumstances. If cotton had not been commercialized for its fiber, the toxicity of gossypol might have remained unknown and certainly would not have been as important. The practical toxicity of a phenol is also related to the amount present and the amount present is not necessarily related to the phenol's frequency of occurrence. Caffeic acid derivatives are commonly present but seldom exceed 5%; true tannin may range from 0 to over 30% of the dry weight in different plants or plant parts. Uncommon phenols are often present in their particularly notorious source in high amounts.

The plants which produce notably toxic phenols are often botanically isolated, divergent, or advanced. The unusual phenol's botanical distribution is a further clue, along with morphology and other composition, to the taxonomic relationships. Specific toxic phenols and phenols with closely related structures are often confined to certain families and not infrequently to certain genera, certain species, or even to certain varieties at certain times. Phenols are considered secondary substances because, except for tyrosine, they do not seem to be essential for life, at least at the cellular level (Neish, 1960; Ramwell *et al.*, 1964). This concept is useful in that it helps explain why plants have been free to develop genetically controlled, chemotaxonomically significant, but highly variable qualitative differences between families. Similarly, the plant is relatively free to respond to environmental and physiological changes by quantitative variation in phenol synthesis. This "secondary" status of phenols, however, should not obscure the fact that they may have important roles in plants partly because they have been free to evolve and to adapt to environment.

Toxicity may, in fact, be a *function* of phenols in plants. There are examples of varieties of a plant species being more or less resistant to pests or pathogens depending upon their content of natural tannin or other phenols (Cruickshank and Perrin, 1964; Goodman *et al.*, 1967; Herrmann, 1962; Kuć, 1966; Pridham, 1960; Rich, 1963). Attack by pathogens may be successfully resisted by rapid production of higher than normal, inhibitory concentrations of phenols. In a few proved cases phytoalexins, so far mostly phenols, specifically inhibitory to plant pathogens are produced in response to attack (Goodman *et al.*, 1967; Cruickshank, 1963). The effect which seems most significant, however, is the apparent employment of localized synthesis of high levels of usual phenols to kill certain plant cells themselves. According to this theory, which seems to have been demonstrated in some instances of hypersensitivity at least, the attack by a would-be parasitic organism triggers the production of chlorogenic acid or other phenol so rapidly and to such a high level that the plant cells quickly die and collapse ahead of the invading organism. A local necrotic spot develops which is walled off, and the would-be parasite dies or is confined to a saprophytic existence in the dead tissue while the rest of the plant remains relatively unaffected. Still other effects of plant phenols which might be interpreted as self-toxicity include the natural inhibitors of seed germination such as coumarins which must be leached or destroyed during a dormancy period before the seed will sprout (Berrie *et al.*, 1968; Pridham, 1960). The ecological relationships which influence competition between root systems of adjacent plants and between roots and soil microflora seem to involve inhibitory effects of lignin degradation products and other plant phenols released into the soil (Flaig, 1967; Hennequin *et al.*, 1967; Kefeli and Turetskaya, 1967; Wang *et al.*, 1967).

The action of the simpler plant and synthetic phenols as antiseptics—i.e., their antibacterial effects as well as animal toxicity—is the most thoroughly studied aspect of phenol toxicology (Von Oettingen, 1949). Essentially every phenolic substance has some antibacterial properties (Jenkins *et al.*, 1957). This generally holds true for both usual and unusual plant phenols although the activity may be of very low order. Commonly, a second hydroxyl group reduces the antibacterial activity. Introduction of alkyl substituents on the ring tends to reduce the animal toxicity and increase the antibacterial effect. Etherification generally decreases toxicity. In fact, animal detoxification of phenols may involve methylation of phenols as well as ethereal sulfate or glucuronoside conjugation (Booth, 1961; Fairbairn, 1959; Williams, 1959). The major point, however, is that phenols, including those of the plant type, are actually broad spectrum, if often weak, toxins to plants, microorganisms, and animals. At high levels even "desirable" phenols may be toxic (National Academy of Sciences, 1966). For example, tyrosine at 5% in a low protein diet for rats produces toxic symptoms in a few days (Bocter and Harper, 1968).

Any phenol may be toxic to any organism under some form of administration. Unadorned, this is basically a naive statement because the same might be said about water. It is true, however, of phenols at moderate levels and under relatively mild conditions of administration. The toxicity of a given phenolic molecule may be related to its reactivity in a nonspecific chemical sense, for example, membrane damaging activity by solvent power of phenol or by acidity of salicyclic acid. It may be related to acute interference with normal biochemical functions of a somewhat general nature such as uncoupling of oxidative phosphorylation, a capability of many phenols. Acute animal toxicity of certain phenols may devolve from interference with catecholamine metabolism and other nerve control mechanisms to produce hallucination, spasm, or convul-

sions. Chronic or long term animal toxicity may take the form of interference with vital substances such as vitamin E, vitamin K, and estrogens. It may be manifested as carcinogenic activity or liver damage of a limited degree of specificity. In any specific instance, operative toxic mechanisms of phenols are often speculative, but phenols do have several toxic capabilities. The particular effect of a given phenol may depend upon a particular stereochemistry and the phenolic hydroxyl may or may not be important. However, phenols have the possibility for more generalized toxicity, and differences in physiological effect may often relate to structure "ornamentation" which confers lipid solubility, prevents or delays detoxification, breaks down into toxic fragments after penetration to a vulnerable site, etc. In a series of substituted phenols, complex mathematical values relating molecular structure, ionizability, and relative lipophilic-hydrophilic character have been linearly correlated with relative toxicity to plants, relative toxicity to bacteria, and activity in uncoupling oxidative phosphorylation (Fujita, 1966).

Common food plants have a limited array of almost universally occurring phenols. Appreciable negative physiological activity of these substances would be surprising. Animals should have evolved means of tolerating them through frequent contact. This appears to be true; the phenols commonly present in plant foods are free of toxicity (Bate-Smith, 1954; Fairbairn, 1959). In view of the general toxic potential of phenols perhaps it would be better to say they are readily eliminated or detoxified by animals. To discuss detoxification mechanisms (Booth, 1961; Fairbairn, 1959; Ramwell *et al.*, 1964; Williams, 1959) would take us too far afield, but since the flavonoids are commonly metabolized in animals to separate phloroglucinol and B-ring derivatives and since the toxic effect of these fragments is fairly well known, probable toxicities sometimes can be predicted. For example, since *d*-catechin, cyanidin, and mavidin are evidently nontoxic, the flavan-3-ol analog of malvidin (which is not known in nature) should be nontoxic.

Conversely, a plant phenol with unusual structural features is more likely to be physiologically active or toxic in animals. Particularly this is true if the unusual structure is such that it would interfere with or prevent the normal detoxification mechanisms or produce toxic fragments. Thus the presence of phenols in all plants and some unusual phenols in a few plants, would appear to have dietary and evolutionary significance when contrasted with, say, the alkaloids which are absent in most food plants and appear generally to be more toxic and not as easily handled by animals in their diet.

The idea that the unusual phenols of plants are more likely to be toxic because animals have had less time to evolve mechanisms to cope with them assumes that the unusual phenol is a relatively recent mutation. That this may not be true is suggested by the fact that the ginkgo tree, redwoods, cyacads, and other "living fossils" tend to be "unusual" in their chemical makeup including phenols. One wonders if seed dispersal and other factors have given a competitive advantage in evolutionary ecology to those plants "pleasing" to animals. Certainly man's influence would have been in that direction even prior to development of agriculture. In a less deliberate sense, animals other than man and birds would distribute seeds in droppings and otherwise aid dissemination of plants which they frequent.

That the phenol content of plants influences selection by animals of their food cannot be doubted. Bate-Smith (Fairbairn, 1959) has emphasized that plant products chosen for exploitation as food are not only edible but also attractive, substantial, and convenient. Leafy foods consumed directly by man are low in lignin, therefore less tough. This choice tends to confine such foods to herbaceous plants with their associated constellation of phenols, notably a low content of condensed tannins, leucoanthocyanidins. Woody plants are a main source of fruit and the fruits are the only parts of such plants commonly eaten. Tannins may be high in the fruit and astringency, bitterness, color, and texture, which are all phenol related, will influence dietary choice among fruits or timing of harvest for a given crop. Certainly a trial food which produced even transiently uncomfortable effects or illness would be shunned as soon as the connection was realized if a choice was possible.

The normal intake level of phenols is quite variable according to diet but may be larger than is commonly recognized. Carnivores would ordinarily have a very low intake of phenols other than tyrosine. Omnivorous animals, like man, may consume considerable amounts of phenols depending upon the food they select or to which they are restricted. For example, red table wines contain about 1500 mg. per liter of total phenols and account for an annual per capita consumption of about 150 grams of "tannin" in some societies. Individuals are known to consume as high as 1000 grams per year from this source. For comparison, the minimum total essential amino acids for adult nitrogen balance is only about 7000 grams per year. Most fruits, some vegetables, and other plant-derived beverages such as tea are often relatively high contributors to the amount of phenols in the human diet.

Herbivorous animals consume massive amounts of phenols. If lignin is considered, phenols are probably second only to carbohydrates as dietary constituents and may approach 20% of the dry weight of the entire food intake. Although lignin is usually considered metabolically inert, recovery from and other tests of "digestibility" indicate often about 10% and sometimes more than half of the lignin fed is solubilized and may be absorbed in ruminants, rabbits, etc. (C.S.I.R.O., 1957; Lenz and Schürch, 1967). The lignin exiting from the gut is chemically different, generally with a lower carbon, hydrogen, and methoxyl content. The content of benzenoid compounds in the urine increases when purified lignin is fed even to dogs. Whether any of the lignin furnishes useful energy to the animal or whether the changes are dependent upon a particular microflora in the alimentary tract are beside the point that a sizable portion of the large amount of phenolic material passing through the animal may be solubilized and absorbed. Of course, lignin fragments and associated phenols as well as most of the flavonoids and other nonpolymeric phenols are able to pass animal membranes and can be absorbed from food.

Before considering some of the interesting specific examples of the toxicity of plant phenols to animals in more detail, let us compare some values for acute toxicities (Table I) (Jenner *et al.*, 1964; Spector, 1956; Stecher, 1968). Such toxicity values are subject to considerable variation depending on the conditions of the experiments and can only be grossly compared when separate studies are involved. However a few interesting relationships seem to be evident. A few substances outside the group

being discussed are included for comparison. Firstly, the acute toxicity to rats of most plant phenols or similar synthetic phenols is rated as slight (LD_{50} in a single oral dose, 500 to 5000 mg. per kg.). A few are moderately toxic (LD_{50} oral, 50 to 500 mg. per kg.), but only a very few approach the highly toxic level (LD_{50} oral, 1 to 50 mg. per kg.), and none is extremely toxic (1 mg. or less per kg.) (Spector, 1956). The estimated comparable amounts for lethal doses in man would be about 250 grams for slightly toxic substances, 30 grams for moderately toxic, and about 3 grams for highly toxic substances. Most phenols are more acutely toxic than ethanol, man's most common intoxicant, much less toxic than strychnine or the "animal" phenols epinephrine or 5-hydroxytryptamine, and about in the range of toxicity of many potent medicinal substances.

The reputations of individual substances for toxicity appears considerably distorted by their individual frames of reference. Toxic phenols have tended to be considered on an individual, ad hoc, or curiosity basis. Rotenone as an insecticide or fish poison is noted for low toxicity to warm-blooded animals. Relative to its toxicity to cold-blooded animals and evidently to other insecticides this is correct, but it is still relatively toxic among the plant phenols for all animals. A synthetic vitamin K is about as toxic on an acute basis as other phenol derivatives some of which are banned from foods. Rutin, a bioflavonoid which is usually considered nontoxic and perhaps a desirable dietary constituent, appears nearly as acutely toxic as dicoumarol, which is noted for slow poisoning. The listed toxicity for quercetin seems unexpectedly high. More recent studies indicate that rutin produces an acute LD_{50} intraperitoneally to rats of 9110 mg. per kg. and no effects when administered orally for 3 months at 850 mg. per kg. per day (Radouco-Thomas *et al.*, 1965). Toxicity in the form of eye cataracts results from contamination of rutin (Nakagawa *et al.*, 1965), and other such problems may produce excessively high toxicity in individual instances. In general, flavonoids of the common types show no acute toxicity at oral doses of about 500 mg. per kg. and intraperitoneal doses of about 50 mg. per kg. (Böhm, 1960). Owing perhaps to the amounts of material required and the costs of obtaining the substances, relatively few studies of lethal oral levels of flavonoids have been reported.

Since microorganisms may not only modify phenols but may destroy them entirely, the microflora of the alimentary tract may considerably reduce the apparent toxicity of orally administered phenols. This would be particularly true of animals adapted to plant diets such as rabbits and horses. Ruminants should particularly profit from this form of assistance considering not only the extensive plant-adapted rumen microflora but also the extra fermentation time allowed in their system. Ordinarily the LD_{50} for rats or rabbits dosed intravenously is about one-tenth the LD_{50} dose orally (Spector, 1956). When the oral toxicity is less than this in proportion to the intravenous toxicity, either greater than usual barriers to absorption into the bloodstream or destruction in the gut, presumably by microorganisms, can be hypothesized. Some phenols fit this one-tenth "rule" and some do not. The high intravenous toxicity for epinephrine and tannin (Table I) are noteworthy. Conversely, some substances seem less toxic parenterally than would be expected from the oral data (eugenol, safrole), presumably owing to lipoid solubilization in the digestive tract.

Table I. Some Approximate Minimum Lethal Acute Toxicities[a] (Mg./Kg. Body Weight; Rat, Oral Except as Noted)

Substance	Toxicity		
Phenol	530	S.c.	400
o-Cresol	1350	S.c.	650
m-Cresol	2020	S.c.	900
p-Cresol	1800	S.c.	500
Anisole	3700	I.p.	500
Catechol	3890	S.c. (rabbit)	225
Guaiacol I.v. (rabbit)	3.7	S.c.	900
Vanillin	1580		
Resorcinol	450		
Hydroquinone	320		
Phlorglucinol		S.c.	1550
Pyrogallol (rabbit)	1100	S.c. (rat)	650
Gallic acid		S.c.	5000
Methyl salicylate	887		
Coumarin	680		
Dihydrocoumarin	1460		
Carvacrol	810		
Thymol	980	S.c.	1650
Anethole	2090	I.p.	70
Eugenol	2680	I.p.	900
Isoeugenol	1340		
Safrole (rabbit)	1950	I.v.	200
Dihydrosafrole	2260		
Methylenedioxybenzene	580		
β-Naphthol		S.c.	2940
Cyclohexanol	2060		
Ethanol	13660	I.p.	5000
Chlortetracycline	3000	I.v.	118
Menadione (mice)	1000		
Serotonin		I.v.	30
Epinephrine	30	S.c.	7
	I.p. 10	I.v.	0.005
Strychnine	16		
Quercetin (mice)	160	S.c.	100
Rutin (mice)		I.v.	950
Lupulone	1800	I.m.	330
Tannic acid (mice)	6000	S.c.	200
		I.v.	80
Rotenone	133	I.p.	5
Dihydrorotenone	330–2500		
Dicoumarol	542	I.v.	52
Gossypolacetate (cat)		I.v.	75
Podophyllotoxin (mice)	90		
Cotoin S.c. (frog)	8		

[a] For clarity, no distinction has been made between LD_{50} (most of the values) and other estimates of typical or minimum lethal doses. S.c. = Subcutaneous, I.p. = intraperitoneal, I.v. = intravenous and I.m. = intramuscular administration.

Too few data were found for very firm conclusions, but a possible relationship between increasing toxicity of phenols to more strictly carnivorous animals can be inferred from acute toxicity data (Jenner *et al.*, 1964; Spector, 1956; Stecher, 1968; Von Oettingen, 1949). The cat seems most susceptible, the dog nearly so, the rat or mouse intermediate, and the rabbit or guinea pig more tolerant. This is not just the effect of internal microbes since parenteral administration tends to show the same relationship. For example, phenol itself injected subcutaneously gave average fatal dose values of 85 mg. per kg. in the cat, 270 in the dog, 290 in the mouse, 300 in the frog, 385 in the rat, 470 in the guinea pig, and 620 mg. per kg. in the rabbit (Von Oettingen, 1949). Admittedly these are highly selected values, but they and many other less extensive comparisons do suggest some lesser resistance of carnivores to phenols of the plant types and may help explain some of the rather large differences in susceptibility to

phenols frequently noted between animal species. The high susceptibility of cold blooded animals like the frog to certain phenols does not carry over to all other phenols. Animal scientists are well aware of differences between domestic animals or even between breeds in susceptibility to toxic reaction from various forages, etc., but often these have not been clarified from a chemical or biochemical viewpoint.

Toxicity in a practical sense is much more involved than is indicated by simple acute lethal potency, of course, and there seems to be no completely satisfactory way to systemize the heterogeneous data available for a brief presentation. A botanical origin, pharmacological effect, biosynthetic relation, or chemical structure approach becomes cumbersome if made complete or adhered to rigidly. Probably selection of examples will be more useful than too great an effort to systemize the data available. However, all systems reach the same focus because a certain uncommon molecular feature produces a certain physiological effect and only certain plants can synthesize phenols with that feature.

Hydroquinone's para dihydroxylation is relatively unusual in plant phenols and is responsible for one type of fatal forage poisoning of livestock which has had considerable local economic impact. Cocklebur (*Xanthium* spp.) seeds contain toxin shown to be hydroquinone and although the spiny burrs are seldom eaten, seedlings still in the decotyledon stage retain toxic levels of the poison (Kingsbury, 1964). The seeds (separated from the burrs) are toxic at about 3000 mg. per kg. of body weight and the symptoms of anorexia, nausea, muscular weakness, convulsions, and death in about three days may be reproduced exactly by hydroquinone administration. All farm animals are susceptible except that poultry present few symptoms other than profound depression. Arbutin, hydroquinone glucoside, occurs in pear tree parts other than the fruit, but does not present a significant hazard. At one time arbutin, probably from bearberry, was used as an orally administered urinary antiseptic. Administration with tannin made it more effective by inhibiting β-glucosidase action on it in the digestive tract (Stecher, 1968).

Salicylic acid is the "plant" phenol responsible for the most actual deaths in humans, primarily through overdose of aspirin by children or suicides. About 200 deaths annually occur in England and Wales from this cause (Dixon *et al.*, 1963). Of course, poisonings from direct consumption of plant material bearing salicylates or related substances like salicin are not a real problem. Although the medical value of these compounds was learned through use of willow bark (*Salix* sp.) and these and *Populus* species produce many chemically interesting variations (Thieme, 1967), we do not usually think of salicylates as plant phenols. The physiological effects of salicylates have been studied in detail (Dixon *et al.*, 1963; Smith and Smith, 1966).

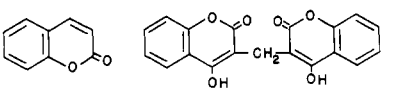
The acute toxic effects often are more related to the strongly acid carboxylic function and effects on acid-base balance than specifically to the phenolic nature. The hydroxyl substitution ortho to the carboxyl is, however, relatively rare in plants and it is significant that this uncommon substitution pattern is involved in these drugs. Vitamin K is helpful in treating salicylate poisoning.

Methyl salicylate exhibits analgesic properties when rubbed on the skin indicating penetration by that route. The efficacy of the methyl ester and the acetate in aiding penetration to a desired cite of action without the strongly irritant effect of free salicylic acid indicates the importance of such factors as blocking groups or lipid solubility on producing different physiological symptoms. Salicylates bind to albumin in the blood and are no longer dialyzable (Moran and Walker, 1968). A strong ionic binding site with pK 8.5 to 9.0 is indicated plus weak sites which are three times as numerous. The mechanism of pain suppression by aspirin may be inhibition of oxidative phosphorylation at specific pain receptor sites (Smith and Smith, 1966).

Salicylic acid is now generally not permitted as a food additive; however, as wintergreen flavoring, methyl salicylate is still used. Chronic toxicity studies for period up to two years have shown no effect in rats fed 1000 p.p.m. of methyl salicylate in their diet or dogs given daily oral doses of 50 mg. per kg. (Hagan *et al.*, 1967). At higher dosages, liver fatty degeneration and weight loss or growth retardation were primary symptoms. With 0.3% acetylsalicylate in the diet of chicks, the growth rate was not significantly affected and anaphylactic response to bovine protein was reduced (Morgan and Glick, 1968).

Coumarin (I) is an ortho hydroxy derivative and might be considered as related to salicylic acid. However, it is also an α-β unsaturated lactone, a structure often physiologically potent. Since it is detoxified in animals by hydroxylation in the 3-, 7-, 8-, or sometimes 5-positions, it would be expected to be more toxic than the usual more phenolic coumarins (Williams, 1959). Open-ring homologs of salicylic acid occur among animal metabolites of coumarin, but analgesic activity of coumarins is doubted (Booth *et al.*, 1959; Soine, 1964). In plants, coumarin and some of its derivatives have inhibitory and regulatory interrelationships with plant hormones; and hydrogenation of the nonaromatic double bond considerably affects activity, at least in certain competitive reactions with gibberellin (Berrie *et al.*, 1968; Khan, 1967; Knypl and Rennert, 1967).

Coumarin itself has toxic action in many animals and the symptoms are manifested in several different ways. It has been considered a narcotic for rabbits, frogs, earthworms, and many other animals, a sedative and



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hypnotic for mice, and a toxin for man or dogs. About 5 grams may kill a sheep and 40 grams a horse, but about 4 grams is required to produce relatively mild toxic effects in humans (Dean, 1952; Sethna and Shah, 1945). Coumarin occurs in a number of natural flavoring materials. It is probably most concentrated in tonka beans, but a number of other odorous products contain it in significant amounts including lavender, lovage, and woodruff. Evidence of extensive liver damage at high dose levels in experimental animals has caused the use of coumarin and tonka beans to be banned as food additives since 1954 (Hazelton *et al.*, 1956; Jacobs, 1963; National Academy of Sciences, 1966). Diets containing 1% coumarin killed rats within about 8 weeks (Hagan *et al.*, 1967). Growth was suppressed and liver damage occurred at dosage lower than 1%. At 1000 p.p.m. of coumarin, in a diet including 3% corn oil and fed for two years, no effect on the rats was detected. Daily oral doses to dogs at 10 mg. per kg.

for nearly a year produced no definite effect; but at 25 mg. per kg., liver damage was marked in one dog, mild in two others. Dihydrocoumarin produced no effect at 1% of the diet fed for 14 weeks lending support to the unsaturated lactone function as at least part of the reason coumarins are unusually active. The sedative and hypnotic effect of coumarins seems particularly related to the unsaturated lactone feature (Soine, 1964).

The coumarins as a group are probably the most widely distributed phenols in plants which have important physiological effects on animals. The coumarins are present (in some species) in decreasing order of frequency in the families Umbelliferae, Leguminoseae, Rutaceae, Compositae, and Solanaceae (Duquenois, 1967). All of these families are considered evolutionarily relatively advanced. Coumarins occur in a few cases and usually with unusual structures in a few lower plants, aflatoxin, for example. Furocoumarins are found in the first three families above plus the Moraceae which are not so advanced, and there are some occurrences of coumarins in other botanically diverse plants. The more complex coumarins are more isolated in occurrence and more likely to be toxic.

The simpler coumarins have been cited as toxic agents in some poisonous plants. Esculetin, 6,7-dihydroxycoumarin, in the form of esculin its 6-glucoside, has been indicated to be the primary toxic agent in a number of buckeyes, *Aesculus* species (Hippocastanaceae) (Kingsbury, 1964). Cattle and other animals may die from consuming leaves or nuts and the nuts are reported to have killed children. Symptoms in cattle included incoordination, twitching, and sluggishness or excitability. However, other coumarins, notably daphnin in *Daphne mezerum*, are now believed not the significant toxin in poisonous plants although formerly so indicated. Esculetin is a potent inhibitor, especially in vivo, of phenylalanine hydroxylase and experimentally induces phenylketonuria (DeGraw *et al.*, 1968). This and other physiological effects including liver damage and capability of involvement with other specific enzyme systems suggest that coumarin in general can have significant toxic action (Dadak and Zboril, 1967; Degkwitz *et al.*, 1968; Feuer *et al.*, 1966). Soine (1964) tabulates 115 coumarins from natural sources including a few from microorganisms and lists 31 reported types of pharmacological and physiological effects on animals. Studies have generally noted a high incidence of physiological effects among different coumarins, but animal genera differ in symptoms and susceptibility. Particularly, high sensitivity exists among fish and snails to both simple and more complex coumarins such as the furanocoumarins (Dean, 1952; Sethna and Shah, 1945; Soine, 1964; Stanley, 1963).

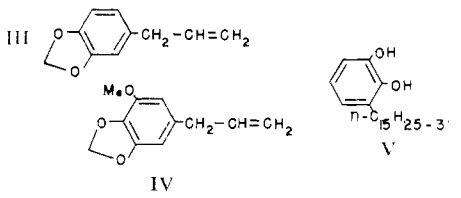
Dicoumarol, 3,3′-methylenebis-4-hydroxycoumarin (II) is a poisonous phenol from a plant source which became a respectable medicine whose gross method of action, if not its specific mechanism, is clear. That prolonged feeding on improperly cured, spoiled hay from sweet clover, *Melilotus officinalis* (Leguminosae), causes a fatal hemorrhagic disease in animals, particularly cattle, and that the active principle is a reaction product derived by mold action from the natural coumarin is now well known (Kingsbury, 1964; Link, 1944; Soine, 1964). Dicoumarol interferes with the action of vitamin K and prevents the synthesis of prothrombin and associated blood clotting factors in the liver. The resultant hypoprothrombinemia inhibits clotting if controlled and produces fatal bleeding if excessive. Initial therapeutic doses are of the order of 200 mg. in humans and reduction in prothrombin clotting time below 20% of normal can be corrected by vitamin K administration. Something more than simple competitive effect is indicated by the fact that relatively large doses of vitamin K are required to reverse the effect of dicoumarol. The drug is apparently stored only in the liver (Wosilait, 1968). It forms complexes with protein in blood (Nagashima *et al.*, 1968b) and the biological half-life increases markedly with increased dosage. Side effects other than bleeding are rare even with prolonged dosage, but owing to the liver storage, the effect on prothrombin level persists for some time.

The structural features which govern antivitamin K anticoagulant activity appear more complicated and interacting than once thought, and although considerable data are available from natural and synthetic analogs, precise conclusions are not yet possible (Soine, 1964). Ayapin, the methylenedioxy derivative of esculetin, is reported to have anticoagulant activity and the addition of aromatic methoxyl substitution to dicoumarol analogs tends to enhance but hydroxyl to decrease activity. Dicoumarol appears to have additional effect as a vasodilator particularly on coronary blood vessels. This effect is pronounced with a number of other coumarin derivatives such as those associated with khellin in *Ammi visnaga*.

Safrole, 1-allyl-3,4-methylenedioxybenzene (III), makes up about 80% of sassafras oil from the root bark of *Sassafras albidium* (Lauraceae). It is also a component in star anise oil, camphor oil, nutmeg, mace, cinnamon leaf oil, and some other essential oils. It was used in small amounts for many years as a flavoring, particularly in root beer. Evidence that it is carcinogenic has resulted in its being banned for food use in the U. S. since 1960 (Hagan *et al.*, 1967; Roe and Field, 1965). Rats died on diets containing 1% and malignant hepatic tumors were found at levels above 0.25% in the diet. Liver damage, including lipoid deposits, but not malignant tumors, decreased from slight to moderate with 0.1% safrole in the diet to very slight at 0.01% or 100 p.p.m. of dietary safrole fed for two years. Daily oral doses to dogs for prolonged periods produced drastic effects including fatty liver damage, but not carcinoma, at 40 and 80 mg. per kg. body weight. At 5 mg. per kg. per day for six years, liver damage was still evident but minimal.

Dihydrosafrole produced much decreased but similar liver damage to safrole. It also produced tumors in the esophagus in 20% of the rats and 5% of the tumors were malignant at a dietary dihydrosafrole level of 0.25% fed over a two-year period (Hagan *et al.*, 1967). In the same tests, *isosafrole*, the 1-propenyl analog, had similar toxic effects to safrole—i.e., liver not esophagus tumors—but required five times as much for comparable severity. Tests with several other essential oil components were more limited in dosage and length. Anethole, *p*-propenylanisole, showed slight microscopic changes in the liver of male rats only, after 15 weeks with 1% in the diet. Similar treatment with dihydroanethole indicated slight osteoporosis after 19 weeks. Eugenol, 1-allyl-3-methoxy-4-hydroxybenzene, or isoeugenol, the propenyl analog, at 1% in the diet of rats for 19 weeks, produced no effect.

The seriously toxic feature of the safrole molecule evidently is the methylenedioxy group, but the degree and locale of the toxic symptoms produced is also considerably influenced by the nature of the hydrocarbon sub-



IV V

stituent. The only metabolite of safrole reported in animals is piperonylic acid (Williams, 1959). Piperonal produced no effect when fed to rats at 1% of the diet for 14 weeks (Hagan *et al.*, 1967). Other methylenedioxy derivatives, notably those in the sesame oil lignan series, have not shown as serious toxicity as safrole even in prolonged feeding (Ambrose *et al.*, 1958; National Academy of Sciences, 1966), although benign liver damage was noted at high levels.

Myristicin, 1-allyl-3,4-methylenedioxy-5-methoxybenzene (IV), is an important constituent of nutmeg oil, nutmegs, and mace from *Myristica fragrans* (Myristicaceae). Nutmeg is the seed kernel and mace the dried aril covering the seed. Consumption of nutmeg in (for a spice) large amounts produces drowziness, stupor, and even death. The volatile oil has much the same but reduced activity and will produce narcosis, delirium, and death. Some of the symptoms of the delirium produced in some people include fairly prolonged or recurrent hallucinations or stupor with short lucid periods between and distorted perception of sight and sound, etc., which resemble the effects of tetrahydrocannabinol or lysergic acid diethylamide. For this reason there has been considerable recent interest in myristicin and nutmeg out of all proportion to the 30 or so known poisoning cases and one documented fatality from this source (Green, 1959; Shulgin, 1966; Truitt *et al.*, 1961; Weil, 1965; Weiss, 1960). A whole nutmeg weighs about 5 grams and contains 5 to 15% volatile oil and 25 to 40% nonvolatile ether extractables. Two nutmegs have produced death when eaten by an 8-year-old child and toxicity requiring medical attention has resulted from smaller doses in adults. Smaller doses do not always produce appreciable toxicity, perhaps partly due to individual sensitivity (Truitt *et al.*, 1961), but also due to variable myristicin content and partial loss of volatiles from the spice.

Nutmeg essential oil has about 4% myristicin, 0.6% safrole, and the remainder is largely terpenes, particularly *d*-camphene. The myristicin fraction from nutmeg, however, is contaminated by about 30% elemicin, 1-allyl-3,4,5-trimethoxybenzene. If the volatile oil was removed, 10 grams of nutmeg residue did not produce hallucination, but still produced intestinal discomfort and affected sleeping (Truitt *et al.*, 1961). Myristicin in 400-mg. oral doses produced "cerebral stimulation" in some people, but much less than 15 grams of powdered nutmeg (which might be expected to contain about 60 mg. of myristicin). It seems clear from animal experimentation that myristicin or a precursor of it is the crucial component for the toxic effect of nutmeg, but it also appears that potentiating, synergistic, or at least additional factors are present both in the volatile oil and in the residue. Although participation by the terpenes and fatty material in absorption and distribution of myristicin is suspected, proof awaits further work.

Myristicin decreased the sleep-inducing effect of phenobarbital in rats. Doses of 50 mg. per kg. intravenously administered to monkeys produced incoordination and disorientation for about two hours. Prior administration of chlorpromazine masked this effect and the effects of morphine were increased in cats by myristicin. Man seems more susceptible to the disorientation effects of myristicin than are many animals, and doses which produce visible behavioral effects in cats appear to terminate in death within a few days from liver degeneration. Favorable effects of myristicin in a few cases of mental illness have been reported. Myristicin potentiates tryptamine induced convulsions, antagonizes the effects of reserpine, and increases brain serotonin, all effects suggesting action as a monoamine oxidase inhibitor. Addition of the elements of ammonia to the side chain of either elemicin or myristicin would produce amphetamines known to be psycotomimetic (Shulgin, 1966; Weil, 1965).

The obvious possibilities that myristicin would mimic safrole as a long-term, low-potency liver carcinogen and that safrole would be a hallucinogen have not yet been studied in detail. Small doses of oils containing safrole have been reported (Von Oettingen, 1949) to produce hallucination and psychic disturbance lasting several days. The trio found in nutmeg, safrole, myristicin, and elemicin occurs also in the oil from *Cinnamomum glandiferum* (Lauraceae) and myristicin occurs with different related compounds in several essential oils from Umbelliferae and a few from Labiatae. Apiol—the 2,5-demethoxy, dillapiol—the 5,6-dimethoxy and the 2,3,4,5-tetramethoxy relatives of myristicin occur with it in oils from parsley, dill, fennel, or related sources (Toth, 1967). These and other variants with three or more phenolic ethers on the ring all, so far as they have been studied, produce toxicity at moderate levels (Weil, 1965).

Urushiol, the active principle of poison ivy, is a mixture of about 2% 3-*n*-pentadecylcatechol (V), 10% of the analogous Δ-8 monoolefin, 64% Δ-8,11 diolefin, and 23% Δ-8,11,14 triolefin (Dawson, 1956; Markiewitz and Dawson, 1965). Poison ivy and its variants, *Rhus toxicodendron*, belong to the largest genus (120 species) of the 60 in the Anacardiaceae, an interesting family from a utility and a phenolic viewpoint (Kingsbury, 1964; Morton, 1961). Most of the Anacardiaceae are tropical and many of them are noted for skin irritating effects. Nevertheless this family inludes the pistachio (*Pistacia vera*), mango (*Mangifera indica*), cashew (*Anacardium occidentale*), and lacquer-producing trees, particulaly *Rhus verniciflua*, which is used in making oriental lacquerware. The saps of many of these plants will oxidatively darken and can be made to polymerize to an inactive form which is no longer an irritant, but in the fresh or incompletely reacted form they are skin vesicants much like poison ivy.

Mango stem sap, which often contaminates the peel and perhaps the peel itself, but not the fruit, produces a similar rash and allergically cross-reacts to a considerable degree with poison ivy (Keil *et al.*, 1946). The cashew "apple" or the fleshy receptacle bearing the nut is readily eaten out of hand, but the shell oil of the nut itself is very irritating, particularly before roasting. The constituents of cashew shell oil include anacardic acids, salicylic acid derivatives (2-carboxy-3-alkylphenols), with a series of C_{13}, C_{15}, and C_{17} side chains with 0 to 3 double bonds (Gellerman and Schlenk, 1968). Anacardic acids also are toxins in the leaves and nuts of *Ginkgo biloba* (not botanically related), but here the location of the unsaturation is evidently different from the 8, 11, 14 cashew pattern.

Anacardic acids make up about 90% of cashew shell "oil." During heating they decarboxylate to give a cardanol (3-alkylphenol) series of compounds. Cardol, analogous 3-alkyl-5-hydroxyphenols, and other substances such as 6-methylcardol are also present in cashew shell liquor (Murthy *et al.*, 1968; Tyman, 1967; Tyman and Morris, 1967). The commercial production of cashew nuts has made cashew shell liquor available in an estimated 15,000 tons annually from India alone (Morton, 1961), and it is used industrially to the degree that contact dermatitis in harvesters and processors must be a problem of some magnitude.

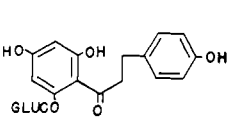
Skin reaction to urushiol and closely related natural and synthetic compounds has an important sensitization-allergy aspect. Babies and others who have never been exposed seem immune to the first exposure to amounts which produce typical reactions in sensitive people. However, a general toxicity also is involved and it has been reported that at elevated levels all persons can be made to react. Upon skin contact with urushiol the reaction does not appear for about four days and the familiar symptoms of redness, itching, blistering, etc., ordinarily subside in less than 14 days. About 1 μg. of urushiols will produce a typical reaction about 5 mm. in circumference on a sensitive individual. The systemic toxicity is relatively low, but serious gastric, intestinal, and nephritic irritation has occurred. Complications from dermal poisoning have produced numerous deaths.

A number of studies have investigated the structure-activity relationships in the urushiol-like series (Baer *et al.*, 1967; Byck and Dawson, 1967; Dawson, 1956) in both human and animal tests. For skin toxicity to nonsensitized guinea pigs an alkyl side chain of at least five carbon atoms is required with toxicity increasing to an apparent limit at about 15 carbons. Branching or cyclization is unimportant and lipoid solubility therefore is more important than shape. Cardol and another meta-dihydroxy analog are nontoxic. A monophenol derivative appears to be as toxic as a catechol derivative provided the structure is capable of being hydroxylated by tyrosinase. The relationship of toxicity and albinism has not been studied, but might explain difficulties in testing urushiol on laboratory rats or mice. Methylation of one phenolic group in 3-pentadecylcatechol reduced considerably the toxicity to nonsensitized guinea pig skin and the second still more but did not entirely eliminate it, presumably because of limited demethylation reactions in the skin.

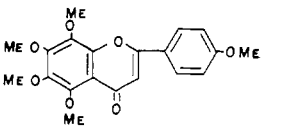
Catechol, in terms of the percentage of guinea pigs becoming sensitive, was as effective in inducing sensitivity to a subsequent homologous contact as were urushiol analogs. The intensity and cross-reactions were affected by the length of the alkyl chains, however, and maximum intensity of sensitivity was produced with 3-undecylcatechol. The sensitivity and toxicity effects are relatively separate as shown by the production of compounds with high activity in one regard and none or little in the other. Urushiols bind to protein in vitro and are rapidly and evidently irreversibly bound very rapidly in skin. This appears to be primarily related to the sensitization phenomenon and represents an amino group covalently linking by nucleophilic addition to the ortho-quinone. This would appear to explain why analogs without activated substitution sites (4,5-dimethyl-3-pentadecylcatechol) and those incapable of direct oxidation to the quinone (6-pentadecylguaiacol) are quite toxic but very poor sensitizers.

Kernels of the "marking nut" (used to mark laundry and thereby causing dermatitis) *Semecarpus anacardium* have been reported to have psychopharmacological effects including improvement of memory, but this lacks satisfactory proof (King, 1957).

Phloridzin (VI), the 2-glucoside of the dihydrochalcone phloretin, occurs in apples and close relatives in parts other than the fruit. It has considerable interest from a botanical viewpoint (Pridham, 1960; Williams, 1966) and because it is one of the few dihydrochalcones known in nature. In early studies in connection with diabetes, it aroused interest from its toxic ability to produce glucosuria in man and animals (Lotspeich, 1960–1; McKee and Hawkins, 1945). Flavanones and chalcones are generally nontoxic like most common flavonoids. Therefore phloridzin is of interest as perhaps the simplest or most nearly "usual" flavonoid analog with generally recognized toxicity. The "toxicity" is of low order, however, 200 to 400 mg. per kg. being commonly used in experimental glucosuria production.



VI



VII

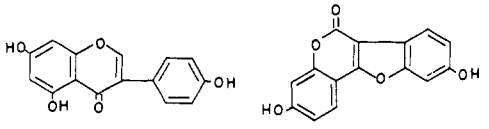
Phloridzin blocks reabsorption of glucose into the blood in the kidney by affecting the epithelial cells lining the proximal convoluted tubules. Prolonged administration leads to hypertrophy of the kidney, but apparently other effects are minimal. Absorption of glucose in the small intestine is also inhibited and the increased glucose absorption occurring in muscle following insulin injection is antagonized. Phloretin is essentially devoid of activity in kidney and intestine but affects glucose absorption by erythrocytes. Absorption of glucose in these cases requires active transport across the membrane. Phloridzin appears to compete by an adsorption process for active sites on the membrane or possibly carrier molecules and is actually extremely potent considering effective concentrations in the target organs.

Reabsorption of fructose or galactose is evidently not affected by phloridzin. The synthetic galactoside of phloretin is much less active, indicating that the 4-position sugar hydroxyl is important in the combining with the active site. If the phenolic hydroxyls are methylated the product is inactive. Uncoupling of oxidative phosphorylation at a specific tissue locus appears to be involved in phloridzin and phloretin action (Deuticke and Gerlach, 1967; Lotspeich, 1960–1).

Tangeretin (VII), 4′,5,6,7,8-pentamethoxyflavone, is uncommon to the extent of the 6- and 8-methoxy groups and is found with nobiletin (additional 3′-methoxyl) in tangerine peel. It is toxic to embryo zebra fish at 0.25 mg. per liter which was only exceeded by podophyllotoxin at 0.1 mg. per liter among compounds tested (Jones *et al.*, 1964). Nobiletin was one-fourth as toxic or less in the same test. Tangeretin was considered nontoxic as were a series of other flavones and flavonones with the more usual hydroxylation pattern in various screening tests including intraperitoneal dosage at 500 mg. per kg. to mice and orally at 1000 mg. per kg. to dogs (Stout *et al.*, 1963, 1964). However, when administered at 10 mg. per kg. per day subcutaneously to rats during gestation, 83% of the litters were born dead or died within three days

without visibly apparent abnormality. Nobiletin is also fungistatic (Ben-Aziz, 1967). The toxicity of these compounds brings to mind the extra toxicity which seems to occur with extra alkoxy groups in the safrole-myristicin series. The toxicity of tangeretin evidently offers no significant natural hazard, but these findings narrowly prevented tangerine peel being introduced as a bioflavonoid source.

Grazing of sheep on subterranean clover (*Trifolium subterraneum*) produces "clover disease," a condition which has caused large economic losses in Australia. The toxic condition is the result of estrogenic isoflavones in the clover. The symptoms include production of lactation in unbred ewes and castrated males, death of castrated males from urinary obstruction, difficult labor, lambing percentages decreased from 80 to as low as 10%, and permanently decreased fertility even when the sheep are removed from the clover. Genistein (VIII) was the first phenol identified as involved in this condition, and other natural isoflavones now known to be estrogenic include biochanin A (the 4'-methoxy analog of genistein), prunetin (7-methoxygenistein), daidzein (5-deoxygenistein), and formononetin (5-deoxybiochanin A) (Bickoff, 1968; Fairbairn, 1959; Moule *et al.*, 1963; National Academy of Sciences, 1966).



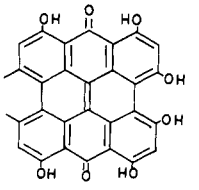
VIII

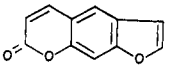
IX

Among 100 species of clover (*Trifolium*), 14 had total isoflavone content comparable to subterranean clover or about 1% of the dry weight of the leaves (Francis *et al.*, 1967). Formononetin is estrogenic in sheep, but less active in mice, compared to daidzein, and the alimentary tract microorganisms are a factor in activation by demethylation and perhaps other mechanisms (Bickoff *et al.*, 1962; Braden *et al.*, 1967; Nilsson *et al.*, 1967). Investigation of other legumes and other estrogen-like responses of animals showed that coumestrol (IX) was an active estrogen from ladino clover, alfalfa, and subterranean clover (Bickoff *et al.*, 1957). It occurs to the extent of about 0.005% of the dry weight of alfalfa and ladino clover, and is about 10 to 30 times as potent as genistein (Braden *et al.*, 1967; Moule *et al.*, 1963; Soine, 1964), depending on the assay used. Other phenolic substances of plants which are estrogenic include miroestrol, a rare but very active pentacyclic monophenol (Cain, 1960) and psoralidin (*Psoralea corylifolia*), coumestrol with 6-C_5H_9 substitution.

The plant estrogens which humans might contact inadvertently seem limited to the isoflavones of soy beans (*Soya hispida*). The amount likely to be consumed in a human diet is too low to be appreciably active. If it were not for the high level in otherwise excellent forage plants and for domestic animals being restricted often to diets almost purely of a certain plant, the same probably would be true for them. Diethylstilbestrol, a synthetic stilbene derivative (but not similar enough to those from conifers to expect them to be active), has two hydroxyls at the extremes of the molecule. It is about 3000 times as active as coumestrol. It appears that activity usually depends on two phenolic hydroxyls or potential hydroxyls a certain distance apart and a relatively planar molecule. Known isoflavones and coumarins with estrogenic activity are limited to those with these features. Synthetic isoflav-3-enes, which mimic diethylstilbestrol even more closely, approach its level of activity.

X





XI

Hypericin (X) and psoralen (XI) represent unusual plant phenols, the former being a dianthrone derivative and the latter a furanocoumarin. Both phenols and their relatives are responsible for toxic reactions making animals hypersensitive to light. Hypericin occurs in species of *Hypericum*, notably St. Johnswort or Klamath weed (*H. perforatum*), while fagopyrin occurs in buckwheat (*Fagopyrum esculentum*). Fagopyrin is similar in structure to hypericin with other groups substituted for the methyl groups and a number of additional variations occur in the same plants (Brockmann, 1957; Clare, 1955; Fairbairn, 1959). The effect on animals may be severe and the invasion of St. Johnswort into grazing land in northern California was becoming a very serious problem until a beetle which selectively feeds on this plant was introduced (Huffaker and Kennett, 1959). The plant, a perennial herb 1 to 5 feet tall, contains hypericin in characteristic glandular dots visible in the foliage and petals (Kingsbury, 1964).

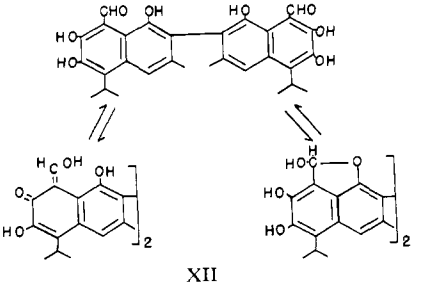
Hypericin remains intact through ingestion, digestion, absorption, and passage through the liver. It may be detected in skin and mucous membranes by direct spectroscopy or its characteristic red fluorescence. Commonly only white skinned animals are poisoned or in those with white spots only those spots are affected (Clare, 1955; Kingsbury, 1964). Unless dosage is very high, completely colored animals are unaffected. Typical photosensitization can be produced in white mice with subcutaneous doses of about 12 to 25 mg. per kg. Cattle or sheep having consumed toxic amounts and appearing well in subdued light are rapidly affected when placed in direct sunlight. Erythema, swelling, extreme itching, serous oozing, and, as the edema subsides, cracking of the skin and necrosis of the skin in spots are symptoms. The animals may appear crazed presumably from the itching and may refuse to eat. Starvation and secondary effects, such as infection from abrasion, lead to most of the deaths. The animals are hypersensitive to touch and to cold water; fording streams may lead to convulsions.

Although hypericin does not seem to be damaging to the liver, decreased effectiveness of liver function may lead to similar photosensitivity from chlorophyll and other substances in the diet. It therefore may be significant that *Hypericum* species seem to have a high level of chlorogenic acid and other phenols which might represent a detoxification load on the liver (Netien and Lebreton, 1964). Photosensitizers evidently act by broadening the wavelengths which produce sunburn and other damage from the normal below 320 mμ to longer ultraviolet and violet light. Free radical formation is involved and oxygen appears required. It is postulated that the localized production of free radicals overloads or circumvents the chain-terminating function of vitamin E and other free radical scavengers and produces cellular disruption and membrane damage, followed by more disseminated effects (Slater and Riley, 1966). Natural melanin granules

seem to *protect* by a similar light absorption-free radical generation mechanism, but the envelopment of these granules in phospholipid membranes apparently confines and dissipates the potentially damaging free radicals in a controlled manner.

Psoralen and related furocoumarins are being studied in some detail because either local application or oral consumption will produce photosensitization. Bergapten (5-methoxypsoralen) has produced a photosensitive dermatitis following applications of perfume containing it, and fowls have had vesicular dermatitis after eating seeds (*Ammi majus*) containing xanthotoxin(8-methoxypsoralen). Cases of dermatitis after skin exposure to celery and certain other juices and sun are believed to be due to this (Soine, 1964). Psoralens, however, have been used medically to stimulate melanin production in humans having vitiligo, a localized "albinism" (Schönberg and Sina, 1948; Trenchi, 1960). The furocoumarins are found in several plant families notably Umbelliferae, Rutaceae, Leguminosae, and Mimosae. Psoralen and very similar structures are the only natural furocoumarins which are photosensitizing, but other toxic effects, fish poisoning, etc., occur in the group.

The relative degree of photosensitizing ability of natural and synthetic compounds and their manner of action have been the subject of several studies (Biswas *et al.*, 1967; Caporale *et al.*, 1967; Pathak *et al.*, 1967; Soine, 1964). There is some disagreement as to the relative activity but it appears that psoralen is one of, if not the, most active, and the entire specific ring system is necessary. Substitution, which has much effect on conjugation or the electronic configuration reduces activity and maximum absorbance at 320 to 360 mμ, is characteristic for active compounds. These compounds emit fluorescence at maxima of 420 to 460 mμ. It has been suggested that emission of this specific light in close proximity to some sensitive component may be important. When we consider the losses involved this seems less likely than a more direct effect of the activation. Methyl substitution at the 3-position but not at others considerably reduces the activity according to some observations. Participation of the 3-position in binding at the active site is suspected. The 4′–5′ double bond in the furane ring is required for activity. *In vitro* studies show a photocatalyzed oxidation of this bond in the presence of flavin mononucleotide. Psoralen combined in vitro, when irradiated, with the pyrimidine bases of DNA. The reactions may be more than localized effects in skin, however, because psoralen causes mobilization of copper from the liver to blood and interactions between the pituitary, melanin formation, and tyrosinase activity (Biswas *et al.*, 1967). Photodynamic action appears to be a more common reaction with phenols than has been generally appreciated; for example, flavones have such action (Nishie *et al.*, 1968).



XII

Gossypol (XII) represents an unusual toxic phenol of very limited occurrence being almost confined to the genus of cotton, *Gossypium* (Malvaceae) (Adams *et al.*, 1960; Bhakuni *et al.*, 1968; Kingsbury, 1964). Gossypol is the predominant yellow pigment which occurs as about 20 to 40% of the substances inside the "glands." These glands are spheroidal bodies about 100 to 400 microns in size, which are visible as dots in the cottonseed kernel. The amount of gossypol is proportional to the number of glands as it does not occur elsewhere in the seed. Cottonseed usually contains 0.4 to 1.7% gossypol, but glandless forms are being bred and *Gossypium* species have from 0.1 to 6.6% of the dry weight of the seed kernel.

The world's production of cottonseed is about 18 million tons and is estimated to contain 55,000 tons of gossypol. All of this is potentially poisoning because both the oil and kernel meal are eaten by man or animals. Fortunately, removal from the oil and causing binding in the meal renders both products nontoxic. If the oil is expressed from the raw kernel it is colorless, the glands are intact, and the meal is toxic to nonruminants. For ruminants it is an excellent feed. If instead the meal is treated with water and cooked with steam, the glands are disrupted and the gossypol partly dissolves in the oil. The oil is readily decolorized and the meal is found to now have most of its gossypol in a bound form which is no longer toxic to nonruminants. With modern processes at least 80% and often 95 to 99% of the gossypol is destroyed, bound, or removed from the meal (Phelps, 1966).

The toxicity of gossypol appears mild on a single oral dose basis; the LD_{50} is about 2400 to 3340 mg. per kg. in the rat (National Academy of Sciences, 1966). Pigs, rabbits, and guinea pigs are more sensitive, chickens about the same. The whole glands appear to be two to four times as toxic as pure gossypol but this may be only lesser inactivation of gossypol en route to a sensitive site. On repeated oral dosage, 10 to 200 mg. per kg. per day is fatal to the dog. With pigs, 0.02% in the diet appears to be the dividing line between toxic and nontoxic (Clawson *et al.*, 1961). This is approximately the level of free gossypol in commercial meal. For safety 0.01% or less free gossypol or not over 9% cottonseed meal in the diet is recommended. At a high level of food protein, gossypol is less toxic.

Pigs fed toxic levels may appear normal for a few weeks to a year, then abruptly begin to gasp for breath and die in two to six days with severe anemia and other complications (Adams *et al.*, 1960; Kingsbury, 1964; National Academy of Sciences, 1966). A common symptom with gossypol in the diet is loss of appetite and weight loss. Gossypol was briefly considered for use in human obesity (National Academy of Sciences, 1966). Hypoprothrombinemia has also been noted as a toxic symptom.

The structure of gossypol is unclassifiable in any usual plant phenol category, evidently because it arises biosynthetically from an isoprenoid route (Heinstein, 1967). The possible equilibrium forms appear to favor strongly the aldehydic form, but to be influenced by solvent and separable by gas chromatography (Raju, 1967). The aldehyde groups are able to react with aniline, ammonia, and more importantly with the free epsilon amino groups of lysine in protein. The bound, nontoxic gossypol is believed mainly this product. Although bound gossypol can be chemically liberated from its Schiff's base combination, this does not happen in digestion. Therefore the loss in toxicity is combined with loss in available lysine as

well (Adams *et al.*, 1960). Gossypol apparently may undergo other types of binding because cooking gossypol in test diets for humans reduced toxicity without reducing protein value or available lysine (Bressani *et al.*, 1964). Simple wetting and drying decreased free—i.e., toxic—gossypol and sugar seemed involved as well as iron and calcium.

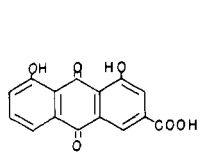
The lack of toxicity of gossypol to ruminants results from indigestible binding of free gossypol to protein which would appear to result from gland mastication and time rather than microorganism effects (Reiser and Fu, 1962). Feeding of iron and of calcium salts with cottonseed meal decreases toxicity of gossypol. It now appears that gossypol binds iron and this is the cause of anemia. If sufficient ferrous ion is added to complex with all the gossypol and still meet the needs of the animal, normal blood conditions will appear, but toxicity will still result. If calcium alone is fed other aspects of toxicity may be prevented, but anemia will result. If both iron and calcium are added to the diet at correct levels normal animals result (Braham *et al.*, 1967). Ferric ion precipitates gossypol, but ferrous ion gives a soluble chelate which is rendered insoluble by calcium (Shieh *et al.*, 1968).

The most worrisome aspect of gossypol toxicity is that gossypol in the body is bound and retained particularly in the liver, kidneys, and spleen of both pigs and trout (Roehm *et al.*, 1967). The tissue-bound level was half as high after less than one-fourth the oral intake. After 12 months of feeding gossypol, shifting to a gossypol-free diet for 10 weeks gave little change in the total bound in the body of trout. Clearly a very difficult compound for the body to detoxify, or eliminate, perhaps because of the lack of acetate or shikimate "handles."

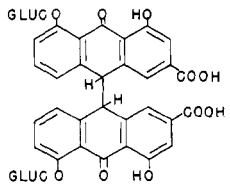
Most of the compounds discussed have been derived at least partly from the shikimic acid biosynthetic route. Anthraquinones of plants have been generally believed to be products of the acetate route, although it appears that that is not exclusively the case and shikimic acid can be involved (Leistner and Zenk, 1967). In addition to hypericin the compounds of this sort which are toxicologically most interesting are the cathartics from *Rhamnus* barks, casara sagrada and frangula; *Aloe* leaf juice, aloin; *Cassia* leaves and pods, senna; and *Rheum* roots, Chinese rhubarb. Cascara sagrada is probably most widely used and an estimated 4 million pounds of this "sacred" bark is collected on the Oregon coast each year, dried, and stored one year before use.

Rhein (XIII) may serve as a model for the anthraquinones which are the basis for the activity of these preparations. The most common structural variants have more reduced substituents in position 3 (aloe-emodin, CH_2OH; chrysophanol, CH_3) or extra hydroxyls (emodin, 6-hydroxychrysophanol). The total anthraquinone content is of the order of 1 to 3% in the natural dry drugs and typical doses would contain about 30 mg. of anthraquinone derivatives. However, anthraquinones from other sources are weak or inactive and gross anthraquinone content is a poor measure of cathartic power in these drugs. Quantitative assay of physiological potency has been a problem, and the mixture of substances turns out to be quite complex as is their chemistry (Fairbairn, 1959, 1964; Kinget, 1968; Lemli, 1967; Stoll and Becker, 1950).

The anthraquinones in these drugs occur as normal phenolic glycosides, and as an O-glycoside of the partially reduced oxanthrone or a C-glycoside of the anthrone form.



XIII

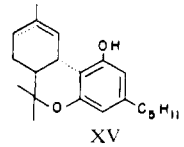


XIV

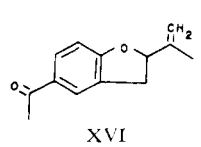
They also occur as homo- and less often heterodianthrones. Sennoside (XIV) is a rhein dianthrone glycoside. The phenolic hydroxyls appear important in activity from work with synthetic substances. In the alpha arrangement one free hydroxyl is inactive, two are active, and three reduce activity unless the third one is not alpha (Fairbairn, 1964). Acetylation causes loss of activity. Glycosidation is important because although anthraquinones produce peristalsis in the large intestine, they do not normally reach it in small dosage unless protected from metabolism by glycosidation. The reduced anthrone and dianthrone forms are more active than the anthraquinone forms. This evidently explains the practice of storage of cascara for a year which is observed to decrease the griping, cramping effects to more gentle action and presumably involves a slow oxidation.

There is, moreover, a synergistic effect when more than one anthraquinone derivative is present. It appears that the action is considerably more than just an "irritant" effect. The specific toxic action seems to be to initiate strong peristalsis in the large intestine. Since the threshold of peristalsis initiation appears to be controlled by 5-hydroxytryptamine (Ramwell *et al.*, 1964), interference with this substance or its oxidation would seem a possible mechanism.

Tetrahydrocannabinol (XV) is the primary active hallucinogen of marihuana or hashish. The chemistry and action of the toxic plant *Cannabis sativa*, hemp, have been reviewed (Mechoulam and Gaoni, 1967; Wolstenholme *et al.*, 1965) and the field is so active that this discussion is limited to a few observations. It is interesting that the plant is related to the nettles, noted for 5-hydroxytryptamine content and for bothering humans. The compound would appear to be a terpene derivative in the biogenetic sense. The structure which is hallucinogenic seems quite specific in that a number of similar compounds which occur with it or have been synthesized, including the unnatural 3,4-*cis* isomer, are low in this activity although sedative or weak antibiotic effect have been noted (Hively, 1967; Mechoulam and Gaoni, 1967; Razdan *et al.*, 1968). Equal activity is found with the double bond in either the 1- or 6-positions.



XV



XVI

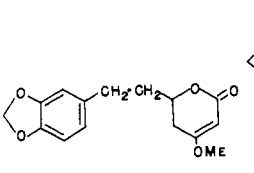
The hallucinogenic dose is about 3 to 5 mg. in humans. Motor incoordination in the dog and abolishment of the blink reflex in rabbits whose eyes are stimulated by a horsehair have been used for assays. The dominant neural effect appears to be impairment of impulse conduction along the presynaptic nerve fiber (Lapa *et al.*, 1968). Marihuana has been reported to increase 5-hydroxytryptamine in the brain, antagonize peripheral effects of 5-hydroxytryptamine, and interfere with peripheral response to acetylcholine and the epinephrines (Bose *et al.*, 1964).

Tetrahydrocannabinol is strongly analgesic at 20 mg. per kg. subcutaneously (Bicher and Mechoulam, 1968). The blink reflex is abolished by about 0.1 mg. per kg. and a natural resin contained the activity equivalent of 10% tetrahydrocannabinol (Valle *et al.*, 1966). A plant resin sample had an acute LD_{50} intraperitoneally in rats of about 800 mg. per kg. and the sublimate produced by smoking only about 3000 mg. per kg. (Wolstenholme *et al.*, 1965). The minimum dose producing measurable incoordination in the same tests was 60 to 95% of the lethal dose in either form.

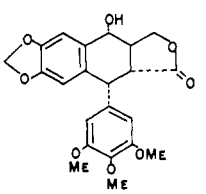
Tremetone (XVI) (Bonner *et al.*, 1964; Christensen, 1965; Kingsbury, 1964) is a poisonous constituent of white snakeroot (*Eupatorium rugosum*) and two other Compositae. The amount of this compound and its derivatives in the plant varies from near zero to about 0.2%. The fresh plant will kill animals when consumed at about 1% of their body weight per day. Human deaths are caused by drinking milk from affected cows. The plant is particularly common on partly cleared land in the east central United States. During the pioneer days in certain areas this plant was the primary cause of human mortality. President Lincoln's mother is said to have died from this "milk sickness."

The structure has some similarity to tetrahydrocannabinol, but the effect on animals has little similarity beyond a certain nervous involvement. Incoordination, apparent muscular stiffness, a reluctance to walk, and trembling spells when the animal is startled or forced to any exertion are symptoms. The compound is retained in the body, produces ketosis, extensive liver and kidney degeneration, and in apparently recovering humans, exertion may bring on fatal relapse even after considerable time.

Dihydromethysticin (XVII) is apparently the most active member of a series of related compounds which have been identified in the intoxicating complex of kava, the Polynesian drink prepared from a shrub, *Piper methysticum*, related to the black pepper plant (Keller and Klohs, 1963; O'Hara *et al.*, 1965; Sauer and Hänsel, 1967). The pharmacology of these compounds is complicated by the fact that they are nearly insoluble and there appears to be synergism and perhaps additional unidentified active components. Mastication, fine grinding, and emulsification increase effective activity, and close filtration is said to remove activity from the beverage. The crude drug produced tranquilization, deep sleep, apparently a pleasant mental outlook and "chaotic" dreams, plus other effects such as temporary limb paralysis.



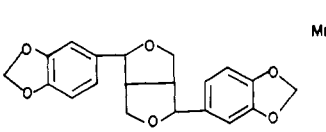
XVII



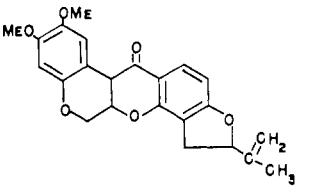
XVIII

Dihydromethysticin in doses of 50 to 200 mg. per kg. orally in oil emulsion produces prolonged sleep and antagonizes strychnine convulsions in mice. The compound and several analogs, including a nonphenolic one, exhibit potent antiserotonin activity. The nonphenol had relatively high antifungal activity (Hänsel *et al.*, 1968). At doses of 500 mg. per day in humans, dihydromethysticin was not effective against schizophrenia, epilepsy, or as a muscle relaxant, but it was a mild tranquilizer. Dry, scaly dermatitis developed in a high percentage of the patients upon continued use. The condition is present ("kavaism") in heavy drinkers of kava. Whether there is a photosensitive aspect to this condition apparently has not been investigated.

Podophyllotoxin (XVIII) is one of a number of very toxic lignan derivatives found in the roots of May apple (*Podophyllum peltatum*) and a small but growing number of other plants (Bianchi *et al.*, 1968; Hartwell and Schrecker, 1958; Kupchan *et al.*, 1967). Members of this group appear to be the most toxic natural phenols of all and it is interesting that other lignans which regularly appear in foods such as Sesamin (XIX) of sesame seed or the antioxidant nordihydroguaiaretic acid are essentially nontoxic. The alcohol-soluble root resin podophyllin was once used as a cathartic, but is too toxic for rational use for this purpose. The fruit of the May apple is mild but pleasing when ripe and so far as is known the plant represents no significant incidence of poisoning in man or animals. Podophyllin is a very powerful cytotoxin which is particularly effective on dividing cells (Di Giamberardino, 1966; Kelly and Hartwell, 1954). Although general toxicity has prevented use of these compounds against clinical cancer they are strongly inhibitory to cancer cells. Certain types of warts are cured by topical application of podophyllin under medical supervision.



XIX



XX

Rotenone (XX) is an isoflavone derivative (Crombie and Thomas, 1967), but represents a group of rotenoids which are of primary interest as insecticides and fish poisons (Crombie, 1963; Feinstein and Jacobson, 1953). They occur in roots of *Derris elliptica* and other related leguminous plants. The general property of higher toxicity to cold-blooded animals than to mammals seems also to operate with tremetone, which is said to be as toxic to goldfish as rotenone, with furocoumarins and with many other phenols. Since rotenone is one of the safest and least residual insecticides applied near or on humans it is often thought of as nontoxic. It is, however, rather toxic among phenols with an LD_{50} oral toxicity to rats of about 130 mg. per kg. and intraperitoneal 5 mg. per kg. Continued application in solution at about 150 mg. per kg. to the skin of rats produced toxicity. Breathing the dust may produce serious toxicity and consumption with oil increases the toxicity. Toxic doses in animals produce incoordination, convulsions, tremors, liver damage, and respiratory difficulties.

Sesamin (XIX), although nontoxic, acts as a synergist for the insecticidal activity of rotenoids. The methylene dioxy grouping is required for activity as a synergist, and a number of substances in addition to sesamin are active. Synergists have juvenile hormone properties in preventing adult metamorphosis in insects (Bowers, 1968), although sesamin has only slight activity.

Rotenone's toxicity appears to depend upon its ability to uncouple or prevent oxidative phosphorylation. This has been known for some time, but the details are now becoming clear (Jeng and Crane, 1968; Palmer *et al.*, 1968;

Papa *et al.*, 1967; Toth *et al.*, 1966). Rotenone evidently blocks NADH oxidation on the oxygen side of the nonheme iron of NADH-dehydrogenase. Under the proper circumstances, menadione opposes and dicoumaro laugments the effect of rotenone in inhibiting respiration.

A number of other examples of physiologically active and potentially toxic unusual plant phenols could be cited, particularly if ferns and lichens were included. However, the list presented is believed to cover all but one of the type phenols important from food poisoning by higher plants or significant toxicity viewpoints.

It was indicated at the outset that we were looking for correlations on the theory that all phenols were likely to be toxic if they could breach the barriers and detoxification mechanisms of the animal. A number of such correlations can be seen. Phenols have an extraordinary solvent power and ability to penetrate the skin, particularly when their fat solubility is reasonably high. The skin toxicity of phenol itself and the topical effectiveness of methyl salicylate, urushiol, podophyllin, and psoralen come to mind. The importance of skin as a target organ for toxicity is indicated by dihydrometysticin as well as by hypericin, psoralens, and urushiols.

A number of suggestive relationships exist between toxicity of plant phenols and the nerve-regulating animal phenols. Cases in point are the proved or inferred mental effects of tetrahydrocannabinol, myristicin, kava constituents, marking nut, and safrole, for example. Also peripheral effects are indicated by salicylates, tremetone, the dianthrones, rotenone, and a number of the others in causing trembling, peristalsis, analgesia, etc. Significantly, one effect of bioflavonoids is to prolong epinephrine action by competitive inhibition of O-methyltransferase (DeEds, 1968).

The mimic-interference which seems to exist between dicoumarol and vitamin K in prothrombin synthesis is also hinted at in gossypol and salicylate toxicity. Activity of genistein and coumestrol seems to depend on fitting a site awaiting natural estrogen. Phloridzin seems to combine its glucose and phenolic properties specifically to prevent glucose transfer. Less direct and perhaps incidental, but certainly intriguing, is the relation between hypericin, psoralen, and tyrosinemelanin metabolism. Even gossypol may affect hair color (Braham *et al.*, 1967), presumably a tyrosinase interaction.

The common observation of synergism or more effect than can be explained by the isolated phenols suggests both a little extra effect from a group of similar analogs, perhaps from the need for a series of adaptive detoxification mechanisms, and also nonspecific toxic aid from other plant phenols which often are present in relatively large amounts but inactive or much less active alone. This picture seems applicable with myristicin, dianthrone cathartics, dihydromethysticin, rotenone synergism, perhaps gossypol, and others.

Animals appear to have more trouble from toxic phenols which are not only unusual among plants but are made by less usual pathways (gossypol, tremetone, tetrahydrocannabinol). The "glands" of gossypol, the similar structures for hypericin, hop resins, and a number of other specialized idioblasts filled with "tannins" in plants suggest that the plant is required to make special arrangements to protect itself from high concentrations of phenols, particularly the more toxic ones. Hashish resin seems to be actually excreted from the plant.

The protection of phenolic groups from the animal's normal detoxification mechanisms until vulnerable sites are reached would seem to be a factor in the extra toxicity of parenteral administration, consumption in oil, probably the frequent but not universal association of methylenedioxy substitution with activity (safrole, myristicin, dihydromethysticin, sesamin), and perhaps the toxicity of the highly methoxylated substances (tangeretin, elemicin, dillapiol).

Finally, and perhaps most importantly, there is the ability of polyphenols to bind metals (gossypol anemia) and to bond by various mechanisms to the macromoles of animal tissue. This seems best illustrated with gossypol although the binding is not phenolic. The substance is not toxic if the binding to protein occurs outside the body, but is bound and "stored" and surely toxic inside the body at least partly because of this type of relatively permanent binding. One wonders if the protective effect of high protein diet is all related to nontissue-protein binding or may partly relate to effects on turnover of tissue protein already bound with gossypol.

Covalent bonding with protein at the activated sites of the urushiol ortho-quinone is evidently involved in poison ivy dermatitis. The acetophenone tremetone may bind by a reaction like gossypol's and tissue storage appears to be a severe aspect of its poisoning. The form of the binding is not clear but podophyllin and psoralen evidently act partly through combination with nucleic acids. The specific membrane adsorption affects of phloridzin may be hydrogen bonding or other weak multiple bonding, but like other phloroglucinol derivatives could involve facilitated covalent bond formation with an electrophilic agent such as a carbonium ion.

These examples seem to add up to fairly convincing presumptive evidence that many of the more toxic phenols act by more than one mechanism and that some of these mechanisms appear to be available to less toxic phenols if natural defenses can be evaded or overloaded. The remaining example of significant toxicity of higher plant phenols mentioned earlier, that of tannin, seems to illustrate this. The tannins of oak leaves, hydrolyzable tannins, are present at levels which lead to major toxic problems in livestock in certain areas. The toxicity of *Quercus havardi* has been most studied and is reported to cause annual losses in excess of $10 million (Camp *et al.*, 1967; Dollahite *et al.*, 1962, 1963). Tannic acid and other oak (hydrolyzable) tannins at high levels produce various toxic effects including intestinal, kidney, and liver damage and anemia. Substances such as iron salts, calcium salts, or high protein which precipitate, oxidize, or bind tannin, when added to the diet aid in preventing this toxicity.

The tannins are present in a number of plant materials at very high levels, often 10% or more of the dry weight. They may be significant in some common feedstuffs such as sorghum grains (Chang and Fuller, 1964) and rapeseed meal (Clandinin and Heard, 1968). The acute oral toxicity of tannic acid is very low and since it is about equivalent to gallic acid, at about 5000 mg. per kg. in rabbits, may represent only complete hydrolysis of gallotannic acid. Continued dosage lowers the toxicity, however, 10 days' dosage gave LD_{50} oral toxicity in rabbits of 3400 mg. per kg. per day. Tannin is much more toxic intravenously, about 80 mg. per kg. LD_{50} in mice (Spector, 1956). This would seem not surprising for a nondialyzable protein

precipitant like intact pentadigalloylglucose tannic acid.

Feeding experiments on chicks with tannic acid and other tannins have shown that at about 0.5% of tannic acid in the diet, growth is depressed and at 5% high mortality occurs (Vohna *et al.*, 1966). Condensed tannins were less detrimental than tannic acid. While these workers found no dietary relationship to methyl donors like methionine, Fuller *et al.* (1967) reported that supplementation with methionine, choline, and arginine reduced the toxicity of 1% tannic acid and completely alleviated the adverse effect of 0.5% tannic acid. This is believed to result from the need for methyl groups for the O-methylation of gallic acid derived from the tannic acid (Potter and Fuller, 1968) as had been shown previously for the rat (Booth *et al.*, 1961) and is a general phenol detoxifying mechanism (Williams, 1959).

The direct absorption of a whole hydrogen-bonding (Singleton, 1967), nondialyzable, protein-precipitating tannin macromolecule seems quite unlikely in the normally functioning animal. However, one of the symptoms of continued tannin feeding is gastritis as well as irritation and edema of the intestines. Under this circumstance it appears that tannin oligomers may be absorbed. The absorption of tannic acid through burned tissue has been known for many years along with the fact that condensed, nonhydrolyzable tannins were less toxic in this situation. The production of liver cancer by application of tannin to burns or by repeated subcutaneous injection has been demonstrated (Bichel and Bach, 1968; Kirby, 1960; Korpassy, 1961). The production of necrosis at the site of injection of condensed tannins but not of tannic acid indicates condensed tannin is more readily bound and being less mobile tends to be less active in liver carcinogenesis.

Tannic acid was introduced about 1946 for use in diagnostic barium enemas to clear away mucus and define colon walls for radiography. It was found in 1963 that this procedure was capable of producing death (McAllister *et al.*, 1963). The incidence of serious or fatal consequences was very low in comparison to the number of treatments, but was higher if the patient was juvenile, repeated enemas were given, or inflammation pre-existed (Janower *et al.*, 1967), and was particularly tragic because it was iatrogenic. The product was quickly banned, and alternative procedures were substituted. The rectal toxicity of tannic acid is about twice that of oral administration (Boyd *et al.*, 1965). The experimental effects of tannin administration include an apparent DNA binding effect in liver cells (Racela *et al.*, 1967) which may relate to carcinogenicity. There is experimental demonstration that hepatoxicity from tannin enemas is dependent upon the time of retention, tannin concentration, and preexisting irritation of the colon (Zboralske *et al.*, 1966).

These results with tannin suggest that on a milder basis some of the observed effects, even desirable physiological effects, of plant phenols may depend upon protein binding and "astringency" (Singleton, 1967) effects. The collagen strengthening, micropore shrinkage, sphincter activating effects of bioflavonoids on blood capillaries, for example (Aichinger *et al.*, 1964; Böhm, 1960; Fairbairn, 1959; Janower *et al.*, 1967), seem related. That the possibility of overloading detoxification mechanisms with nontoxic plant phenols exists, but that the margin of dietary safety is large is indicated by the fact that 2.5% bioflavonoids in chick diets for 8 weeks produced no detrimental effects, but 5% produced reduction in growth and feed utilization without producing gross evidences of tissue damage (Deyoe *et al.*, 1962).

The potential importance of abnormal routes of administration of phenols as well as potentially important effects of degradation products of plant phenols is indicated by studies showing that many simple phenols repeatedly painted on skin increase the incidence of cancer, but only following triggering doses of carcinogens (Boutwell, 1967; Roe and Field, 1965). This would seem to have a bearing on foods and other products where pyrolysis of phenols and coproduction of carcinogenic hydrocarbons could occur such as in smoking (Boutwell, 1967; Fiddler *et al.*, 1967; Kaiser, 1967). Ferulic acid, for example, decomposes thermally to 4-vinylguaiacol and a number of other products.

In summary, plant phenols include a number of substances which are toxic and in certain circumstances do cause great losses. The symptoms and reactions involved seem to indicate a more coordinated picture than is commonly recognized. It appears that a number of the relatively common chemical and biochemical properties of phenols are involved in producing toxicity. While most common plant phenols are essentially nontoxic, their potential toxicity is worthy of consideration if many are combined, consumption is high, or unusual methods of administration are contemplated.

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