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DIETARY TANNINS AND SALIVARY PROLINE-RICH PROTEINS: Interactions, Induction, and Defense Mechanisms

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INTRODUCTION

About four years ago our two laboratories (then in the Department of Biochemistry at Purdue University) had a merger of research interests. Butler and coworkers were investigating the interactions of various proteins with tannins and had reported that proteins high in proline (or hydroxyproline) such as collagen have the highest affinities for tannins (42). Carlson and coworkers had just sequenced the first proline-rich protein (PRP) cDNA (102), part of the tissue-specific multigene family in rat parotid and submandibular glands, which is dramatically induced by isoproterenol (73, 74). The logical experiment was attempted; rats injected with isoproterenol to induce PRPs were fed high-tannin sorghum with the expectation that the PRPs could "neutralize" the well-known detrimental effects of tannins in the diet. This part of the experiment produced the expected results, but control rats fed high-tannin sorghum showed unexpected changes. The parotid glands enlarged about fourfold and there was a dramatic increase in PRPs within three days (54). Tannins in the diet mimicked the effects of isoproterenol by inducing the PRP multigene family. Even more striking, the rats showed an initial loss of weight for three days, and only when PRPs were synthesized did the animals gain weight at essentially the normal rate.

Tannins ingested in large amounts (72, 91), used as treatment for burns (54) and as adjuvant for barium enemas (84), can cause carcinomas, hepatotoxicity, and apparently other pathological and toxic problems. We believe that the PRPs in saliva constitute the first line defense against tannins ingested. Proline-rich proteins comprise about 70% of the total protein in human saliva. For convenience, this review is in two parts, the first part on tannins and the second on PRPs. The authors trust the readers to integrate the two.

TANNINS

Tannins are complex phenol-rich polymers found in many foods (Table 1). The amounts vary widely with cultivar and with cultivation and processing conditions. The common occurrence of tannins in foods reflects their widespread distribution in plant materials, but also is due to the human palate's "taste" for tannin (72). It is otherwise difficult to understand the broad dietary appeal of tannin-rich but nutrient-poor foodstuffs such as tea (62), and the prevalence of the betel quid, extraordinarily rich in tannins, in Eastern cultures (83). Common use of the betel quid is suggested by Duke (28): "Chief use of betel nut is as a breath-sweetening masticatory, enjoyed for centuries by about *one-tenth* of the human population." Antinutritional effects have been associated with dietary tannins, and there is particular concern

about the nutritional consequences when the tannins are in foods that are dietary staples or are otherwise utilized intensively.

Chemistry

Tannins are known historically as plant-derived materials that when applied to animal hides "tan" them into leather. The polyphenolic nature of these materials has sometimes led to applying the term "tannin" carelessly to any polyphenolic plant component (48). As with many naturally occurring polymers, a rigorous chemical definition of tannins is difficult. The capability for strong interactions with proteins such as collagen was likely significant in the use of tannins for burn treatment (54) as well as for tanning leather. Here we adopt a definition similar to that of Gupta & Haslam (37): tannins are water-soluble phenolic metabolites of plants with a molecular weight (MW) of 500 or greater and with the ability to precipitate gelatin and other proteins from aqueous solutions. This definition, however, excludes certain of those phenolics that bind strongly to proteins without precipitation. Tannins are considered secondary metabolites and irregularly distributed substances that have no specific metabolic function. Physicochemically, tannins are complex polymers conveniently divided into two major types, condensed and hydrolyzable tannins.

Table 1 Representative tannin-containing foods[a]

Beverages	Fruits (especially unripe)
Red wine	Banana
Tea	Persimmon
Cider	Apple
Coffee	
Cocoa	
Beer	
Cereals	**Berries**
Sorghum	Strawberries
Barley	Red currants
	Blueberries
	Raspberries
Legumes	**Other**
Fava beans	"Pan" (betel quid)
Pinto beans	Chewing sticks (Nigeria)
Common beans	Condiments
Cowpeas	

[a] Table 1 is adapted from M. L. Price and L. G. Butler, 1980. Tannins and nutrition. *Bull. #272*. Agric. Exp. Station, Purdue Univ.

Condensed Tannins

Condensed tannins are polymers of flavan-3-ols (Figure 1*a*) linked through acid-labile carbon-carbon bonds (Figure 1*b*). In hot, strong, mineral acids, condensed tannins are oxidatively depolymerized (not hydrolyzed) to anthocyanidin pigments (Figure 1*c*) and other less well-characterized products. This conversion to anthocyanidins is the basis for designating condensed tannins as proanthocyanidins, and is the basis of a convenient method of detection. Proanthocyanidins differ in their degree of polymerization from dimers to polymers of hundreds of flavanol units; however, most are in the range of 4 to 40 units (97). Because of their limited solubility and extractability, the larger condensed tannins (>10 units) may have little nutritional significance. Proanthocyanidins also differ in the position (C4 to C8 vs C4 to C6) and stereochemistry of their interflavan bonds (12). Although they are generally assumed to be linear polymers, there is some evidence for branching (60). Virtually all flavanol units of proanthocyanidins are hydroxylated at positions 5 and 7 on their A ring (Figure 1*a*), but they differ in the degree of hydroxylation of their B ring; the catechol pattern of cyanidin is most common (23).

Hydrolyzable Tannins

Hydrolyzable tannins, or tannic acids, are composed of gallic acid (Figure 1*d*) or its condensation product ellagic acid (Figure 1*e*) esterified to the hydroxyl groups of glucose. Additional depsidically linked galloyl groups are usually present (Figure 1*f*)(48). The distinction between condensed and hydrolyzable tannins is somewhat blurred by the occurrence, especially in tea, of hybrid molecules containing both galloyl groups and flavan-3-ol units (87). The gallic-acid-containing tannins are alkali labile and are hydrolyzed by gastrointestinal esterases or by tannin acyl hydrolase (E.C. 3.1.1.20), which is secreted by certain fungi, yeast, and bacteria (25).

Assays

Many different procedures, none of which is entirely satisfactory, have been used to estimate tannins. The lack of standards, especially for condensed tannins, is a major problem. Commercial reagent-grade tannic acid is often used as a standard for hydrolyzable tannins and for protein precipitation assays. Assays for condensed tannins have been standardized with quebracho tannin, which is easily purified from a readily available crude extract (5). Too often, assays such as the Folin-Denis (32) and Prussian blue (79), which detect all phenolic groups (and other reductants such as ascorbate), are used. Such assays may give little quantitative information about tannin content. However, selective extraction procedures combined with these nonspecific assays greatly enhance quantitation (79).

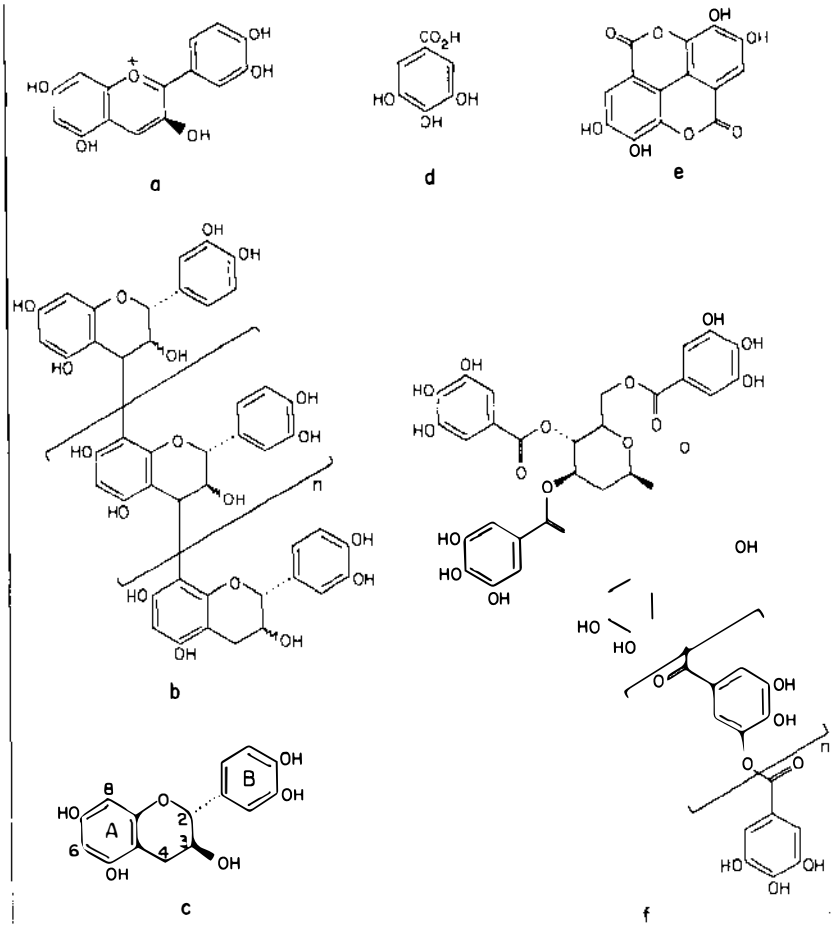

Figure 1 Tannins and tannin components. (*a*) Flavan-3-ol; (*b*) basic structure of a condensed tannin; (*c*) anthocyanidin; (*d*) gallic acid; (*e*) ellagic acid; (*f*) basic structure of a hydrolyzable tannin.

Condensed tannins can be assayed as proanthocyanidins by their oxidative depolymerization to anthocyanidin pigments, promoted by Fe^{+3} in hot acid (78). This assay is readily adapted to measure leucoanthocyanidins (95). Condensed tannins are usually assayed by reaction with vanillin. This assay is conveniently standardized with catechin if purified tannin standards are not available, but catechin standards seriously overestimate tannin content. Replacing vanillin with other aromatic aldehydes (82) and changing the solvent (20) makes the assay more sensitive and selective. Appropriate controls are needed to correct for absorbance by pigments (81). Hydrolyzable

tannins are not detected by these assays, and reliable chemical assays specific for hydrolyzable tannins have not been developed.

Following the lead of Bate-Smith (13), researchers have reported several assays dependent on tannin binding to protein. Generally these assays do not distinguish between condensed and hydrolyzable tannins. An assay for protein-precipitable phenols (40) gave good correlations with nutritional quality of sorghums (19). Use of a plant protein assay (59a) more closely simulates conditions within plant tissues. A convenient gel assay for protein precipitants such as tannins has been reported (39). Protein precipitation assays are facilitated by use of a protein labeled with either a radioisotope (41) or a blue dye, which minimizes interference from plant pigments (5). Soluble tannin-protein complexes can be measured in competition assays (14) in which formation of such complexes diminishes the amount of radioisotope or dye-labeled protein precipitated by a fixed amount of tannin. Attempts to assay tannins by inhibition of a standard enzyme preparation have been unsatisfactory (19).

In summary, no assay for tannins provides an unequivocal value for tannin content. Tannin levels of plant cultivars vary greatly with stage of development, from location to location, and from year to year. Most procedures measure tannins indirectly in an extract of the plant material; such methods obviously measure only *extractable* tannins, although this is seldom acknowledged. For condensed tannins, the amount of material difficult to extract or unextractable may be greater than the amount of readily extractable tannin (37). Even with optimized procedures, there is no assurance that all tannin has been extracted. Claims regarding the absolute amount of tannins present should be considered with skepticism. Statements like "peanut skins contain *x*% tannin" have no significance.

Biological Roles and Dietary Effects

PLANTS There is no consensus on the origin and purpose of plant secondary metabolites in general and tannins in particular (14). Although they may be regarded as metabolic waste products (47), there is little doubt that at times the presence of tannins genuinely benefits the plant. The astringency that tannins contribute to unripe fruits, for example, results in their avoidance by herbivores until the seeds are mature and ready for dispersal. Vervet monkeys select foods at least partially on the basis of the lowest tannin content (98). In the cereal crop sorghum, tannin content of the grain has been positively correlated with resistance to bird depredation (94), fungal molds (45), anthracnose (68), greenbugs (27), and preharvest seed germination (44). Tannin-free cultivars are more vulnerable and can be reliably produced only where these pests are not a major problem.

In addition to plant defense, tannins function more generally as allelochem-

icals; they can influence other organisms even after the plant that produced them is no longer living. Tannins in decaying plant material associate with proteins, membranes, and cell walls and inhibit their degradation (17). In this way tannins may exhibit ecological effects by controlling rates of nitrogen release and buildup of organic matter in the soil, thus affecting growth rates of other plants and indirectly the corresponding animal and microbial population as well (50).

DIETARY EFFECTS As expected from their effectiveness as plant defense chemicals, tannins exhibit a variety of toxic or antinutritional effects when present in the diet. While much work has been done with insects, coverage here is limited to vertebrates. We have recently reviewed the effect of sorghum tannins (21).

As defense chemicals, tannins cause avoidance leading either to diminished food consumption or to selection of other foods. Food consumption in rodents is often depressed, especially with foods containing relatively high levels of hydrolyzable tannins (5%) (52, 69, 93). Condensed tannins at the levels usually fed do not diminish feed consumption (61, 88) and may even increase it (57). Highly adapted animals actually prefer tannin-rich diets (9). Antinutritional effects of dietary tannin are not explained by reduced food intake (36).

Tannins depress the growth rate of young rats (30), hamsters (H. Mehansho, J. Rogler, L. Butler, D. M. Carlson, unpublished information), mice (7), swine (67), and chicks (30). In most cases there is a corresponding decrease in feed utilization efficiency. In humans, tannins reportedly decrease protein utilization (49). Tannins increase the amount of fecal material and fecal nitrogen (30, 69) of endogenous origin (35), perturb mineral absorption (69), and diminish total body sodium (33). The effects of tannins in ruminant nutrition are complex but perhaps less severe than in nonruminants (55). Beneficial effects of tannin reported in ruminants include bloat suppression (86) and protection of dietary protein in the rumen (100).

MECHANISM OF ACTION Tannins are generally large molecules avidly bound to other materials. It is generally assumed they are not absorbed, and that their effects are confined to the digestive tract. Since tannins strongly inhibit digestive enzymes in in vitro assays, the antinutritional effects of dietary tannin are often related to inhibition of digestion. However, digestive enzymes appear not to be inhibited in vivo by dietary tannins (69, 70). More importantly, fecal nitrogen is increased from endogenous origins (35), most likely from tannin-induced and tannin-binding salivary proline-rich proteins. The mechanism by which dietary tannins exert their antinutritional effects may have been wrongly attributed to inhibition of digestion (69).

Hydrolyzable tannins are likely degraded by esterases to gallic acid, which is absorbed and can produce toxic effects such as fatty liver (35), and which depresses rat growth rates similarly to tannic acid (52). Condensed tannins may cause similar physiological responses. Chicks on certain diets high in tannin develop leg abnormalities including outward bowing of the legs with swelling at the hock joint, and may be unable to stand erect (29). This condition, which affects the organic matrix of the bone, is alleviated by "detoxification" of the tannin (80). Chicks on diets of high-tannin sorghum develop elevated levels of liver microsomal UDP-glucuronyltransferase, an enzyme involved in metabolic detoxification of phenolic materials (89). Hamsters are unusually sensitive to tannins; rats and mice readily adapt to condensed tannins at levels that are lethal to hamsters in three days, too short a time for starvation due to interference with digestion (65) to be a major factor.

TANNIN-IRON INTERACTIONS Essentially all dietary iron for the third-world population is of plant origin, or nonheme iron (71). Poor absorption of iron is an important problem since the bioavailability of nonheme iron is influenced by other dietary components. In the past decade, tannins have been identified as potent inhibitors of nonheme iron absorption. The effects of tea on iron absorption was first reported by Disler et al (26). As compared to water, drinking tea reduced the adsorption of ferric chloride by 16% and ferrous chloride with ascorbic acid by 20%. Addition of milk failed to alleviate the tea-mediated inhibition of iron absorption. Meat, like ascorbic acid, is known to enhance iron absorption, but tea markedly inhibited iron absorption from hamburger (43). There are several additional accounts of tannins (or tea) affecting iron availability or absorption (24, 34, 66, 85).

Interactions of Tannins and Proteins

The capacity to precipitate proteins is a part of our definition of tannins. However, as mentioned, not all associations between tannins and proteins result in precipitation. Assay methods that detect soluble tannin-protein complexes may define tannins in broader terms.

ASSAY SYSTEMS Assays of phenolic oligomers of hydrolyzable tannins binding to bovine serum albumin showed that the affinity for penta-*O*-galloyl-β-D-glucose was greatest (63). Conformational flexibility in the polyphenolic ligand contributed to the strength of binding. A competition assay, similar to that used to study antigen-antibody interactions, has been applied to protein-tannin complexes (42). A protein labeled with radioisotope (41) or with an intensely absorbing blue dye (5) is mixed with an amount of tannin that precipitates 60–70% of the labeled protein. In separate samples competing protein is mixed with the labeled protein before adding the tannin.

CHARACTERISTICS OF TANNIN-BINDING PROTEINS Condensed tannins differ in their relative affinity for various proteins by almost four orders of magnitude (42). A protein with high affinity such as gelatin is selectively bound by tannin in the presence of a 100-fold excess of another protein such as lysozyme. In a survey of condensed tannins purified from four different sources binding to six different proteins, only soybean trypsin inhibitor showed no detectable affinity to two tannin samples (6).

General characteristics of proteins with high affinity for tannins are large size, open loose structure, high proportion of hydrophobic amino acids, and high proline content (42). Proteins with lowest affinity for tannin are generally small, compact, and cross-linked with disulfide bonds. The characteristic that best correlates with affinity for condensed tannin is proline content (38). The importance of proline is presumably due to its inability to fit into the α-helix, which leads to a loose, open structure readily accessible to tannins and to the formation of hydrogen bonds with the phenolic groups of tannins (42). Plant proteins such as prolamines have a high affinity for condensed tannin. The haze-forming proteins that bind polyphenols in beer, the prolamine (hordein) fraction of barley (4), are rich in proline.

Interactions between tannins and proteins, and the role of proline in such interactions, led to the discovery of unusual proline-rich tannin-binding proteins in the fungus *Colletotrichum graminicola*. These proteins appear to function in the defense of the fungi against the effects of tannins and other polyphenols. The fungus, which causes the disease anthracnose on cereal crops, produces its spores in a water-soluble mucilage containing a proline-rich glycoprotein that has a very high affinity for condensed tannin (75). In the absence of this glycoprotein fraction, germination of the spores is strongly inhibited by a variety of phenolic materials, including tannins. The use of proline-rich tannin-binding proteins as a defense against tannins may be a common strategy among organisms living in a tannin-rich environment or, as seen with the salivary proline-rich proteins, consuming tannin-rich materials.

CARBOHYDRATE EFFECTS Considerable specificity and significance has been imputed to tannin-carbohydrate interactions (103). The most comprehensive studies of tannins binding to carbohydrates have been performed with fragments of hydrolyzable tannins bound to solid absorbents (63). Binding of tannins to soluble carbohydrates is either difficult to study (63) or is likely too weak to detect (6).

The affinity of the carbohydrate components of glycoproteins for tannins is of particular interest. The catalytic activities of two glycoprotein enzymes, yeast invertase and *Aspergillus flavus* tannase, are quite resistant to inhibition by condensed and hydrolyzable tannins, respectively (92). Binding was not measured, but the carbohydrate was proposed to serve as a barrier against

tannin binding to the protein. The failure of condensed tannin to precipitate bovine submaxillary mucin has been ascribed to carbohydrate on the protein (51). Whether the carbohydrate moieties of the proline-rich fungal glycoproteins have a role in tannin binding is not known.

We recently made a systematic study of the effects of the carbohydrates on a salivary proline-rich glycoprotein (GP66sm) for the affinity with two condensed tannins (7). The deglycosylated protein had the same relative affinities for both sorghum and quebracho tannin, which was 11-fold greater than the affinity of serum albumin. The intact glycoprotein GP66sm had much greater affinity for the tannins than did the deglycosylated protein, and this was 4-fold greater for sorghum tannin and 8-fold greater for quebracho tannin. Removal of sialic acid gave affinities intermediate between the intact protein and deglycosylated protein. No detectable affinity to tannin was observed for the oligosaccharides isolated from the glycoprotein. In summary, the carbohydrate components of GP66sm cause tannins to bind more avidly and confer specificity. The glycoprotein-tannin complexes are more soluble than tannin complexes with deglycosylated protein.

PROLINE-RICH PROTEINS

Background

The first observations that human saliva contained proteins high in proline, or the so-called proline-rich proteins (PRPs), were reported by Mandel, Thompson & Ellison (59) and by Levine, Weill & Ellison (56). A glycoprotein (or glycoproteins) was obtained and the amino acid composition (mol %) was Pro, 34; Gly, 21; Glu, 21; Asp 7. Aromatic and sulfur-containing amino acids were not detected. Subsequently, Bennick & Connell (16) and Oppenheim, Hay & Franzblau (76) isolated and partially characterized a series of PRPs from human parotid saliva that, with one possible exception, did not contain carbohydrate. Amino acid compositions of these PRPs from both laboratories were essentially identical, and were very similar to those reported earlier (56, 59). PRPs in human saliva are constitutive and make up about 70% of the total protein.

As so often happens, we became interested in PRPs through a happenstance. Isoproterenol, a β-agonist, was known to cause hypertrophy and hyperplasia of rat salivary glands (18, 90). Since the inherited disease cystic fibrosis is classified as a "generalized exocrinopathy," and in many cases the salivary glands are enlarged, it was proposed that the isoproterenol-treated rat could be a model system for studying the pathobiochemistry of cystic fibrosis. While this proposed model system is likely not true, studies of the biochemical changes in rat salivary glands initiated by isoproterenol treatment showed unusual changes in protein synthesis (31). An acidic glycoprotein was dramatically induced in rat parotid glands, which contained phosphate and the

following amino acid composition (mol %): Pro, 30; Gly, 17; Glu, 19; Asp 12, but no sulfur-containing or aromatic amino acids. Subsequent studies with rats (73, 74) showed that a family of proteins was induced by isoproterenol treatment, and that most members of the family were nonglycosylated basic proteins containing about 45 mol % Pro. We now consider these proteins to be a first line defense against the detrimental effects of tannin in the diet.

Molecular Biology

The dramatic alterations in protein synthesis prompted us to use cell-free translation analysis to evaluate the potential changes in PRP mRNAs. After four days of isoproterenol treatment, striking quantitative and qualitative changes in PRP mRNAs were observed by translation patterns (101) and by dot-blot analysis (2). PRP mRNAs increased from nondetectable amounts to constitute over 60% of the total parotid gland mRNAs (2). The first total sequence of a PRP cDNA clone was reported by Ziemer et al (102), and the mRNA that encoded the acidic proline-rich glycoprotein first identified by Fernandez-Sorensen & Carlson (31). This rat PRP cDNA clone (pRP33) was used to select partial PRP gene sequences from a human genomic library (11) and to probe DNA Southern blots of hamster:mouse hybrid cell lines to establish that the PRP gene family in mouse is located on chromosome 8 (10).

Currently, PRP cDNAs from mouse (22), rat (102), human (58), and monkey (77) and PRP genes from mouse (1), hamster (3), and human (53) have been sequenced. A common characteristic of all proteins encoded by these cDNAs and genes is that they are composed of four distinct regions; a signal peptide, a transition region, a repeat region, and a carboxyl-terminal region (1, 3, 22, 102) (Figure 2). Comparisons of the signal peptides and repeat regions shows striking homologies of amino acid and nucleotide sequences for all proteins from every species (1, 3, 22). In contrast, the transition regions and carboxyl-terminal regions are quite diverse (3), which poses an interesting problem in evolution.

Recently, we demonstrated that AtT20 cells (2) and PC-12 cells (P. Wright, D. K. Ann, D. M. Carlson, unpublished results) transfected with either mouse or hamster PRP genes are dramatically induced to synthesize PRP mRNAs by either isoproterenol or cyclic AMP, plus theophylline. Preliminary experiments using 5'-upstream deletions have tentatively identified the cAMP-inducible sequence in the mouse gene MP_2 as (−639)ATGTAACAGTCA(−628). The gene organizations of mouse, hamster, and human are compared in Figure 3.

Functional Aspects

Proline-rich proteins in human saliva may be involved in calcium binding, hydroxylapatite binding, formation of acquired dental pellicle, and agglutination of oral bacteria (15). Recently we presented evidence that a primary role

Figure 2 Amino acid sequence of the proline-rich glycoprotein GP66sm as derived from the sequence of the mouse PRP gene MP_2.

of PRPs in saliva is to bind polyphenolic compounds such as tannins and tannic acids, and that feeding tannins to rats (64) and mice (7) mimics the effects of isoproterenol. However, unlike with isoproterenol treatment, no changes were observed in the submandibular glands (64). Amino acid analyses, electrophoretic patterns, and cell-free translations of mRNAs all confirmed that the same families of PRPs are induced by feeding tannins. As mentioned previously, binding curves for PRPs and tannins showed affinities about 10-fold greater than for bovine serum albumin and tannins. A loss of body weight was observed for about three days during feeding of tannin (7, 64). Initiation of weight gain of animals fed tannins was coincident with maximal stimulation of PRP synthesis (7, 64). Subsequent experiments have clearly shown that the initial weight loss is caused by tannins, that tannins and dimers of tannins and tannic acid (but not the monomers catechin or gallic acid) induce the morphological and biochemical changes in the parotid glands, and that the production of PRPs "neutralizes" the detrimental effects of tannins (H. Mehansho, T. Asquith, L. Butler, J. Rogler, D. M. Carlson, unpublished information).

A different picture is presented by hamsters. Hamsters respond to isoproterenol by increasing their synthesis of PRPs (H. Mehansho, L. Butler, J.

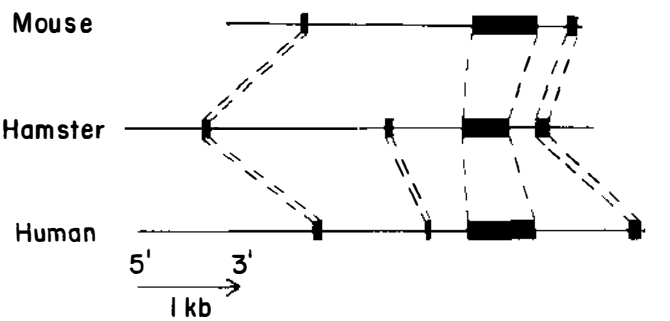

Figure 3 Comparison of the exon-intron organizations of PRP genes from mouse (MP_2), hamster (H29), and human (PRH1). Exonic regions are indicated by the darkened areas.

Rogler, D. M. Carlson, unpublished information). However, the level of glutamine is higher (mol %) in some proteins than proline; e.g. protein HP43a contains (mol %) Gln, 34; Pro, 22; Gly, 20. There is little, if any, hypertrophic response. Feeding tannins has essentially no effect on the salivary glands, and PRPs *are not* induced. Hamsters fed a diet containing 2% tannin lose weight for about three days, as do rats and mice, but then an unusual growth inhibition is observed. Hamsters maintained on a 2% tannin diet fail to grow and even at 60 days are essentially the same body weight as at three days. When the diets were switched, the experimental animals gained weight at almost the normal rate for young hamsters, while the control animals, now on a 2% tannin diet, lost about 20% of their weight. In about 30 days, both groups of hamsters were close to the same weight. Clearly, the detrimental effects of tannins are reversed by the induction of PRPs in rats and mice, but hamsters are unusually susceptible to tannins. In fact, increasing the tannin content of the diet to 4% by adding quebracho tannins has no effect on rats and mice, but this increase in tannins is fatal to hamsters, with most animals dying within three days.

SUMMARY AND FUTURE CONSIDERATIONS

The role of the salivary proline-rich proteins as a "first line of defense" against the detrimental effects of tannins in the diet deserves further study. The expression of the PRP multigene families is regulated by isoproterenol undoubtedly by modulating the levels of cyclic AMP through activation of β-receptors. Preliminary results with cell transfections suggest that isoproterenol may also have a direct action on the PRP genes, or may effect other secondary messengers. Similarly, tannins stimulate β-receptors, likely by some indirect mechanism, since the effects of tannins in the diet are blocked by the β-antagonist propranolol when also fed as a dietary component.

Tannic acid is reported to be a hepatotoxic agent; in rare instances it has led to death in humans after being administered in enemas used for preparing patients for roentgenologic examination (84). In 1963, tannic acid may have been used as an adjuvant for more than 25% of the barium enemas administered in the United States (84). In reports on the toxic effects of tannic acid in barium enemas (46, 84, 99), eight deaths were attributed to multiple tannic acid enemas, with patients dying in two to seven days after the enemas. The livers showed evidence of centrolobular hepatic necrosis similar to that described by Wells, Humphrey & Coll in 1942 (96) when patients died three to five days after tannic acid treatment for extensive burns. Unusual hypersensitivities to tannin and tannic acid in hamsters are also demonstrated by the unusual growth inhibition and toxic effects; about 75% of the animals died within three days after being fed a 4% tannin diet. Neither gross morphologic nor histologic evidence for abnormalities was observed in various tissues of hamsters fed a 2% tannin diet, however (W. Reed, unpublished information).

The molecular basis for the binding of tannin with proline-rich proteins is being investigated. Proline-rich proteins have no apparent stable conformation as determined by circular dichroism and NMR studies (H. Mehansho, B.-L. Kim, D. M. Carlson, unpublished information). Interactions of tannins (condensed tannins and tannic acid), and of various oligomers of each tannin fraction, with PRPs and various peptides and glycopeptides of each PRP, especially from the repeat region, need to be investigated. Effects of varying temperature, pH, buffer, salts, and other ligands should be studied by ^{13}C and ^{1}H high-resolution NMR.

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