# Influence of Dietary Vitamin E and Selenium on Metabolic Activation of Chemicals to Mutagens

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When rats were fed a basal vitamin E (VE) deficient and low-selenium (Se) diet with or without 100 ppm of VE or 2 ppm of Se supplementation, the ability of liver S-9s to mutagenically activate benzo[a]pyrene (BP) was not appreciably altered, although differences in the activation of 2-aminofluorene and 2-aminoanthracene were observed. Aroclor treatment caused a reduction in the mutagenic actiation of aryl amines in all dietary groups while a distinct increase in BP activation was seen. The activity of aryl hydrocarbon hydroxylase was significantly higher in the liver S-9s of VE- and Se-supplemented rats and was markedly increased in all dietary groups as a result of Aroclor treatment. Hepatic GSH S-transferase activity but not the Se-dependent GSH peroxidase activity was increased following Aroclor treatment. The results do not suggest a direct relationship between the enzymatic alterations caused by dietary antioxidants and the ability of hepatic S-9s to mutagenically activate procarcinogens.

Many chemical carcinogens are biologically inactive as parent compounds but can be activated by microsomal enzymes to electrophilic metabolites that may react with the cellular nucleophiles to produce biological effects (Conney et al., 1977; Weisburger, 1979). Metabolic activation of chemicals is generally accomplished by the cytochrome P-450 linked enzyme systems that are located in the microsomal membrane fraction of the cells. Many dietary components, e.g., proteins, lipids, vitamins, etc., have been shown to profoundly influence the activities of the microsomal enzyme systems involved in the activation and detoxification of xenobiotics (Conney et al., 1977; Wade and Norred, 1976; Zannoni and Sato, 1976). By virtue of their ability to alter the enzymatic activities of the tissue, the dietary constituents may in turn influence the ability of the cells to produce reactive intermediates from chemicals and hence their metabolic activation to mutagens.

Vitamin E (VE) has been shown to reduce the binding of benzo[a]pyrene (BP) to nuclear macromolecules (Matsuura et al., 1979) and BP-induced chromosomal aberrations (Smalls and Patterson, 1982). In addition, it has also been reported to influence the 3-methylcholanthrene-induced tumorigenesis (Haber and Wissler, 1962) and the formation of tumors by transplanted murine sarcoma cells (Kurek and Corwin, 1982). Selenium (Se) intake has been shown to be negatively correlated to cancer incidence in humans (Schrauzer et al., 1977; Shamberger and Willis, 1971), and the dietary selenium reduces chemically or virially induced tumorigenesis in animals (Greeder and Milner, 1980; Griffin and Jacobs, 1977; Harr et al., 1973; Jacobs et al., 1977). Furthermore, the possible role of free radical lipid peroxidation in mutagenesis and carcinogenesis has received renewed attention in recent years. Both VE and Se are known to protect cellular constituents against lipid peroxidation tissue damage and have been found to inhibit mutagenesis in vitro (Mukai and Goldstein, 1976; Shamberger et al., 1979). The studies reported here were carried out to determine if dietary vitamin E and Se are capable of altering the metabolic activation potential of selected chemicals. In addition, the influence of dietary VE and Se on the activities of hepatic aryl hydrocarbon hydroxylase (AHH), glutathione (GSH) peroxidase, and GSH S-transferase was also examined.

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## MATERIALS AND METHODS

**Experimental Animals, Dietary Regimens, and Tissue Preparation.** One-month-old Sprague-Dawley rats were maintained on a basal VE-deficient and low-Se diet that consisted of the following (%): Torula yeast, 30; sucrose, 59; tocopherol-stripped lard, 5; salt mixtures, 5; vitamin E free vitamin mixture, 1, as in Schwarz and Fredga (1969) and supplemented with either none, 100 ppm of VE (as *dl*-α-tocopheryl acetate), or 2.0 ppm of Se (as sodium selenite) ad libitum. The basal diet contained 0.02 ppm of Se as determined spectrofluorometrically (Spallholz et al., 1978). After 11 or 19 weeks on the respective diets, the animals from each dietary group were injected intraperitoneally with Aroclor 1254 in sesame oil (500 mg/kg of body weight) or with sesame oil only. Aroclor treatments were given to rats to determine the inducibility of cytochrome P-450 linked hepatic enzymes in different dietary groups. Five days after Aroclor injections, animals were sacrificed. There were no animal mortalities during the experimental period. Livers were excised and homogenates were prepared in 0.05 M Tris buffer containing 1.15 M KCl, pH 7.5. The homogenates were centrifuged at 9000*g* for 30 min and the supernatants (S-9) immediately frozen and stored at −80 °C until used for enzymatic and mutagenicity studies, which were conducted within 1 week from the day of sacrifice.

To assess the nutritional status of VE, blood samples from each animal were collected to measure the degree of erythrocyte hemolysis (Draper and Csallany, 1969), and the level of plasma VE was determined as described previously (Chow et al., 1971). Se status of the animals was monitored by measuring the activity of plasma Se-dependent GSH peroxidase (Lawrence and Burk, 1976).

**Mutagenesis Assays.** Activation of benzo[a]pyrene (BP), 2-aminoanthracene (2AA), and 2-aminofluorene (2AF) to mutagens was measured by using *Salmonella typhimurium* tester strain TA98 by the agar overlay technique of Ames et al. (1975). The tester strains were kindly provided by Dr. B. N. Ames (University of California, Berkeley). Supernatants from each group of animals were used in the S-9 mixture for metabolic activation of test compounds. Each plate received supernatants containing 2–3 mg of protein. Tests were performed on three concentrations of each compound and at least three

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Table I. Dietary Vitamin E and Activities of Aryl Hydrocarbon Hydroxylase, GSH S-Transferase, and GSH Peroxidase in Rat Liver[a]

| dietary vitamin E, ppm | Aroclor treatment | aryl hydrocarbon hydroxylase, nmol $min^{-1}$ $g^{-1}$ | GSH S-transferase, $\mu$mol $min^{-1}$ $g^{-1}$ | Se-GSH peroxidase, $\mu$mol $min^{-1}$ $g^{-1}$ |
|---|---|---|---|---|
| 0 | − | 13.3 ± 0.7[a,b] | 11.4 ± 0.4[a] | 1.03 ± 0.18[a] |
| 100 | − | 29.9 ± 3.2[b] | 12.1 ± 0.5[a] | 0.96 ± 0.16[a] |
| 0 | + | 203.0 ± 7.3[c] | 16.4 ± 0.7[b] | 0.94 ± 0.20[a] |
| 100 | + | 187.5 ± 3.1[c] | 14.4 ± 2.1[a,b] | 1.04 ± 0.21[a] |

[a] One-month-old male rats maintained on a basal VE-deficient and low-SE diet with or without VE supplementation for 11 weeks were treated with either Aroclor or placebo 5 days prior to sacrifice. Four to six animals in each group. [b] Mean ± SEM. Means that do not share the same superscript are different significantly ($P < 0.05$).

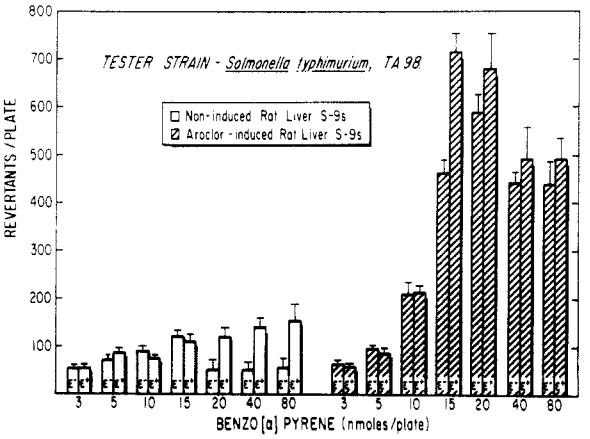


**Figure 1.** Metabolic activation of benzo[*a*]pyrene to mutagens by liver S-9s from vitamin E deficient and supplemented rats.

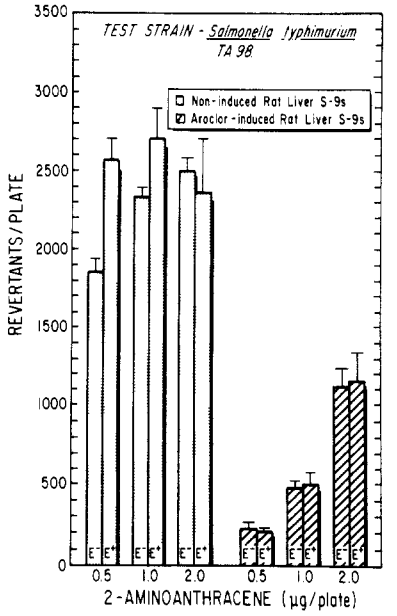


**Figure 2.** Metabolic activation of 2-aminoanthracene to mutagens by liver S-9s from vitamin E deficient and supplemented rats.

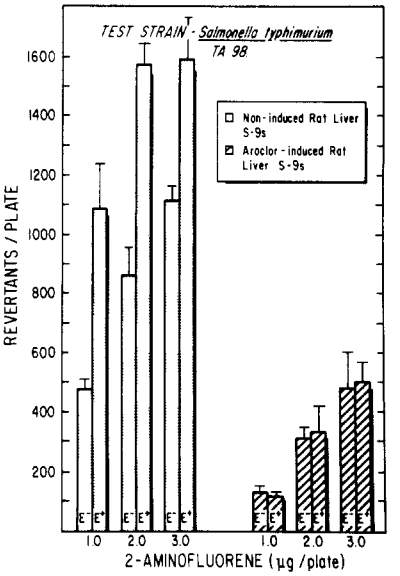


**Figure 3.** Metabolic activation of 2-aminofluorene to mutagens by liver S-9s from vitamin E deficient and supplemented rats.

plates were used for each concentration.

**Enzyme Assays.** AHH assays were peformed by using the radiometric procedure of Van Cantfort et al. (1977), which measures the overall conversion of [$^3$H]BP to its polar metabolites. Aliquots of 9000*g* supernatants (S-9s) were incubated at 37 °C in the presence of [$^3$H]BP and cofactors. The reaction was terminated by adding a 1 M aqueous KOH/dimethyl sulfoxide mixture (15/85). The unmetabolized BP was removed by three extractions with hexane, and an aliquot of the aqueous phase was counted for radioactivity to determine the total amount of BP converted to polar metabolites. GSH *S*-transferase activity was measured by using 1-chloro-2,4-dinitrobenzene as the substrate (Shreve et al., 1979). The activity of Se-dependent GSH peroxidase was measured by using $H_2O_2$ as the substrate (Lawrence and Burk, 1976).

The statistical significance of differences between mean values was determined by using the Student's *t* test at the 95% confidence level.

## RESULTS

**Vitamin E.** At the time of sacrifice, the animals maintained on the basal diet, with and without VE supplementation, for 11 weeks weighed 222 ± 17 and 203 ± 7 g, respectively. Aroclor treatment 5 days prior to the sacrifice resulted in a reduction of body weights to 196 ± 8 and 172 ± 11 g in vitamin E supplemented and non-supplemented animals, respectively. Erythrocyte hemolysis values averaged over 90% and below 5%, and plasma VE levels were 0.45 and 4.45 mg/mL, respectively, for the VE-deficient and -supplemented groups. These values did not change significantly by the Aroclor treatment.

The AHH activity of S-9s from different diet and treatment groups is given in Table I. Animals maintained on the basal diet had significantly lower hepatic AHH activity than those in the VE-supplemented group. The enzyme levels in Aroclor-treated animals were increased by 15- and 6-fold, respectively, in VE-deficient and -supplemented rats. However, statistically significant differences were not found between the two dietary groups when Aroclor-induced hepatic AHH levels were compared. The activity of GSH *S*-transferase was significantly higher in the liver of Aroclor-treated rats maintained on the basal diet but not in the VE-supplemented group. Dietary VE had no significant effect on the activity of this enzyme. The activity of Se-dependent GSH peroxidase was not significantly affected by either Aroclor treatment or dietary VE.

Table II. Dietary Selenium and Activities of Aryl Hydrocarbon Hydroxylase, GSH S-Transferase, and GSH Peroxidase in Rat Liver[a]

| dietary selenium, ppm | Aroclor treatment | aryl hydrocarbon hydroxylase, nmol $min^{-1}$ $g^{-1}$ | GSH S-transferase, $\mu$mol $min^{-1}$ $g^{-1}$ | Se-GSH peroxidase, $\mu$mol $min^{-1}$ $g^{-1}$ |
|---|---|---|---|---|
| 0 | − | 16.9 ± 2.7[a,b] | 12.1 ± 0.8[a] | 0.78 ± 0.16[a] |
| 2.0 | − | 29.4 ± 2.5[b] | 11.6 ± 1.7[a] | 5.41 ± 0.67[b] |
| 0 | + | 118.0 ± 8.5[c] | 18.4 ± 1.4[b] | 0.79 ± 0.16[a] |
| 2.0 | + | 95.0 ± 12.6[c] | 16.4 ± 2.4[b] | 5.16 ± 0.56[b] |

[a] One-month-old male rats maintained on a basal VE-deficient and low-Se diet with or without VE supplementation for 19 weeks were treated with either Aroclor or placebo 5 days prior to sacrifice. Four to six animals in each group. [b] Mean ± SEM. Means that do not share the same superscript are different significantly ($P < 0.05$).

Mutagenicity tests with *Salmonella typhimurium* strain TA98 showed that enzyme preparations from all of the four groups were generally able to activate BP, 2AA, and 2AF to mutagens, although each to a different degree (Figures 1–3). Aroclor treatment of animals increased the activation potential of hepatic S-9s for BP in each group. However, a significant reduction in the mutagenic activation of 2AA and 2AF occurred when supernatants from Aroclor-treated rats were used from either of the two dietary groups of animals. Such a reduction ranged from 2- to 10-fold depending on the concentration of the test compound used on the plate. Enzyme preparations from noninduced rats maintained on the basal diet were generally unable to activate BP to mutagens. S-9s from VE supplemented rats activated BP to a limited extent. S-9s from noninduced rats of both dietary groups were very efficient in activating aryl amines to mutagens (Figures 2 and 3). VE supplementation of noninduced animals increased the ability of their enzymes to activate 2AF.

**Selenium.** At sacrifice, the body weights were 322 ± 15 and 422 ± 24 g, respectively, for animals maintained on the basal and Se-supplemented diets ($P < 0.001$) for 20 weeks. Aroclor treatment caused no significant reduction on the body weight of either dietary group of animals. The effect of dietary Se and Aroclor treatment on hepatic levels of AHH, GSH S-transferase, and Se-dependent GSH-peroxidase expressed as units per gram of tissue is given in Table II. Animals maintained on a Se-supplemented diet for 20 weeks showed significantly higher AHH activity than those fed the basal diet. Treatment of animals with Aroclor increased the enzyme activity by severalfold. No significant differences were observed in the induced levels of AHH between the rats of the two dietary groups. Similar results are indicated when the data are expressed as units per milligram of protein.

As expected, low dietary levels of Se caused a significant reduction in the level of hepatic Se-dependent GSH peroxidase (Table II). Compared to the Se-supplemented groups, a decrease of GSH peroxidase activity by about 85% was observed in rats maintained on basal diets. The enzyme activity was not significantly affected by Aroclor treatment in either of the dietary groups. GSH S-transferase activity was also significantly increased in the liver of Aroclor-treated rats for either the basal diet (52%) or the supplemented diet (41%). Dietary Se had no significant effect on the activity of this enzyme under the experimental conditions.

The metabolic potential of liver enzymes for activation of three test compounds to mutagens is shown in Figure 4. Mutagenicity tests were performed in the presence of S-9 mixtures from different rats in each group. Distinct differences in the ability to mutagenically activate the test compounds were observed between liver preparations from control and Aroclor-treated animals. S-9 mixtures from control animals were more active for 2AA and 2AF while preparations from Aroclor-treated rats were more efficient at BP activation. Enzymes from the Se-supplemented group showed higher activity for 2AF but lower for 2AA. However, dietary Se status did not produce a distinct influence on the activation potential of hepatic supernatants.

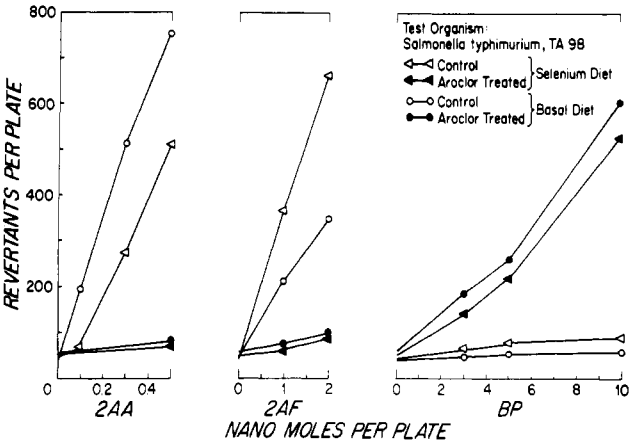


**Figure 4.** Metabolic activation of 2-aminoanthracene, 2-aminofluorene, and benzo[*a*]pyrene by liver S-9s from selenium-deficient and -supplemented rats.

## DISCUSSION

Many chemical carcinogens require metabolic activation to produce biological effects. The cytotoxic, mutagenic, and carcinogenic effects of a number of chemicals are believed to result from their enzymatic conversion to reactive intermediates that may either interact with the cellular macromolecules to produce adverse effects or become detoxified by a variety of cellular enzymes to inactive metabolite conjugates (Weisburger, 1979). The microsomal mixed-function oxidases constitute an important class of enzymes that are involved in the metabolic conversion of a variety of chemicals (Conney et al., 1977).

Aryl hydrocarbon hydroxylase is a cytochrome P-450 linked microsomal enzyme system that plays an important role in the metabolic activation of polycyclic aromatic hydrocarbons. A number of studies have reported the effect of dietary VE and Se on the cytochrome P-450 linked enzymes in the rat liver (Carpenter, 1972; Horn et al., 1976; Burk and Masters, 1975; Diplock, 1974; Gairola and Chow, 1980, 1982; Chen et al., 1982a,b). Our results are in agreement with other reports that VE and Se deficiencies depress hepatic AHH activity in untreated rats but have no effect in Aroclor-treated rats. A recent study (Chen et al., 1982a) reported similar effects for VE deficiency in rats but found no effects of Se deficiency on AHH. The induced AHH levels in phenobarbital-treated rats were, however, found unaffected by deficiency of VE or Se, an observation in agreement with our results of Aroclor-treated animals. Similarity of low dietary Se and VE effects on hepatic AHH activity suggests a common mechanism for these two dietary antioxidants.

The role of GSH S-transferase in the conjugation of potentially harmful electrophilic metabolites of xenobiotics to endogenous GSH is well documented (Jacoby, 1977). Our studies indicate that the activity of hepatic GSH S-transferase was not significantly affected by either dietary VE or Se, although the enzyme activity was significantly increased by Aroclor treatment in all dietary groups (Tables I and II). The activity of GSH S-transferase has been reported to be significantly increased in the livers of rats maintained on a Se-deficient diet (Lawrence and Burk, 1978).

Prohaska and Ganther (1977) have identified a GSH peroxidase activity in the purified GSH S-transferase from rat liver that is independent of Se and is involved in the detoxification of cellular organic peroxides but not hydrogen peroxide. Such activity in liver was found to be significantly increased as a result of dietary Se depletion or Aroclor treatment (Chow and Gairola, 1981). On the other hand, the Se-dependent GSH peroxidase activity in livers was not affected by either dietary VE or Aroclor treatment. However, as expected, the Se-dependent GSH peroxidase activity was markedly decreased in livers of rats fed the Se-deficient diet. GSH peroxidase and metabolically related enzymes have been implicated in the metabolism and detoxification of cellular hydroperoxides (Chow, 1979).

Evaluation of the liver homogenates for their ability to mutagenically activate selected test compounds did not exhibit any remarkable differences between VE- or Se-supplemented rats and those maintained on the basal diet, although minor differences were present. In general, an elevation or decrease in the AHH activity positively correlated with the ability of S-9s to activate BP to mutagens while an inverse relationship was observed between AHH and the mutagenic activation of 2AA and 2AF. Aroclor treatment of animals that increased the AHH activity resulted in reduced capacity of hepatic S-9s to activate aryl amines. While the mechanism of this differential effect is not yet known, these findings are in agreement with those of Schutt et al. (1978).

In vitro addition of Se to the reaction mixtures in the Ames test has been found to reduce the mutagenic activation of various compounds (Jacobs et al., 1977; Rosin and Stitch, 1979; Martin et al., 1981). Recent studies by Schillaci et al. (1982) have shown that Se supplementation in drinking water or diet decreases the mutagenic activation of DMBA by S-9s obtained from rats treated with Aroclor at doses of 20–100 mg/kg of body weight. These workers also found that a reduction in the number of DMBA-induced revertants is not observed in the presence of S-9s from rats given Se in drinking water and treated with 500 mg of Aroclor/kg of body weight, a dose generally employed for obtaining fully induced S-9s for use in the Ames test. Similarly, in our studies significant differences is mutagenic activation of another polycyclic aromatic hydrocarbon, BP, were not noticed when S-9s from control and Aroclor-treated (500 mg/kg) rats maintained on the basal diet with or without Se supplementation were used. However, we did observe small differences in the activation of aryl amines (2AA and 2AF) by S-9s from untreated rats fed basal diet.

Aroclor treatment of both basal and Se-supplemented groups significantly reduced mutagenic activation of aryl amines by S-9s. In rats maintained on Se-supplemented drinking water, Marshall et al. (1979) have reported a shift in the metabolism of (2-acetylamino)fluorene from the activating N-hydroxylation to detoxifying ring hydroxylation pathway. Using bacterial mutations as the end point, we did not observe any clear-cut differences in the ability of S-9s from rats fed low-Se or Se-supplemented diets to generate mutagens from 2AA or 2AF.

The role of diet/nutrient as a modifier of the enzymes involved in the metabolism of drug and environmental chemicals is well-known. Dietary VE and Se have been shown to alter the activities of enzymes involved in the activation and detoxification of xenobiotics. Whether such alterations in the activities of these enzymes can be directly associated with the mutation induction in bacterial tests performed in vitro is not clearly understood at this time. The differential effect of dietary antioxidants on the enzymatic alterations in various tissues (Gairola and Chen, 1982) and also on the covalent binding of carcinogens to cellular DNA in different species (Chen et al., 1982a,b) further complicates the interpretation of these results. More studies are needed to better understand the role of dietary VE and Se in mutagenesis and carcinogenesis.

**Registry No.** VE, 1406-18-4; AHH, 9037-52-9; GSH S-transferase, 50812-37-8; GSH peroxidase, 9013-66-5; BP, 50-32-8; 2AA, 613-13-8; 2AF, 153-78-6; Se, 7782-49-2; Arochlor 1254, 11097-69-1.

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End of Symposium

# REVIEW

## Tannins: Their Adverse Role in Ruminant Nutrition

Ravindra Kumar*[1] and Manohar Singh

This review attempts to provide a current summary of literature concerning the chemistry of tannins and their various adverse effects upon ruminant feed quality. These include the chemical nature of hydrolyzable and condensed tannins, their occurrences in numerous feeds and fodders, their interaction with proteins, and subsequent effects upon voluntary feed intake, dry matter and protein digestibility, and rumen metabolism. In addition, the processing of tannin-rich ruminant feed for their increased utilization is also discussed.

Tannins present in many important forages, several agricultural wastes, agroindustrial byproducts, and fodder tree leaves not only affect the feed quality adversely but also cause toxicity. Several episodes of loss of livestock from easting oak acorns have occurred in Europe (Wolter, 1974) in the American southwest (Sandusky et al. 1977; Keeler et al., 1978), and in South Africa (Naser et al., 1982), owing to the fact that oak leaves and twigs are eaten heavily at times when little else is available. Further, the inexorable scarcity of livestock rations in developing countries has made it obligatory to incorporate tannin-rich feeds in the livestock ration. Enormous work has been done to overcome the ruminants' nutritional problems associated with the presence of a high quantity of tannins in feeds and fodders; however, the literature is scattered and the last comprehensive review on the subject by M. N. McLeod appeared in 1974. Therefore, the present review attempts to compile and summarize the recent studies on the adverse effect of tannins upon ruminants' feed quality and rumen enzymatic reactions and various methods for their removal from ruminants feed. The beneficial effects of tannins such as the prevention of bloat and protection of protein against rapid ruminal degradation deserve a review of their own and are not included here.

**Chemistry of Tannins.** The term tannin referred originally to substances with the ability to tan leather. It is now generally used to include any naturally occurring compound of high enough molecular weight (500–3000) and containing a large number of phenolic hydroxylic groups (one to two 100 molecular weight) to enable it to form effective cross-links with protein and other molecues (Swain, 1979). The hydrolyzable and condensed tannins are two groups of these compounds widely distributed in the plant kingdom, which may be differentiated by their structure and reactivity toward hydrolytic agents (Freudenberg, 1920; Haslam, 1966). The chemistry of the two groups have been extensively reviewed (Swain, 1979; Haslam, 1981).

The potential livestock feeds rich in hydrolyzable tannins are green pods of *Ceratonia siliqua* (Joslyn et al., 1968), leaves of *Quercus robus* (Feeney and Bostock, 1968), acorns of *Quercus incana* (Vijjan and Katiyar, 1973), acorns of European Oak (*Quercus pedunculata*) Singleton, 1981), and deoiled sal (*Shorea robusta*) seed meal (Kumar,

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