With equal quantities of protein applied to each well, only the total buffered saline-soluble fraction (T) and the isocratic pool (1) for Yellow Creole (A) showed hemagglutination. For Huffman (B), major hemagglutination activity occurred in pooled fraction 2 followed by weaker activity in pooled fraction 3. Results indicated that at least 200 μg of protein was required for visible agglutination. These results showed correlation between antifungal effects and lectin activity for Yellow Creole in solid agar assays; in A & M medium, however, the major antifungal effects occurred in individual fractions corresponding to pooled fraction 2. In Huffman, no correlation between lectin activity and fungal growth inhibition (solid or liquid media) was observed.

The specific activities of lectins in separated fractions of maize proteins were reported recently (Newburg and Concon, 1985). Although some activity was detected in saline-soluble extracts, most of the total activity was concentrated in the alkali-soluble glutelins. Vast literature on plant lectins have described their structures, sugar specificities, and practical uses as analytical tools; however, their physiological function that might benefit the general health of plants remains unclear (Etzlor, 1981; Brambl and Gade, 1985). One study postulated that wheat germ agglutinin inhibits chitin synthesis and spore germination in certain fungi (Mirelman et al., 1975).

These preliminary results clearly demonstrate inhibition of normal metabolic pathways in *A. flavus* growth induced by polypeptides from maize and might explain differences in aflatoxin levels observed under field conditions. Physicochemical properties such as molecular size, antigenic/electrophoretic behavior, and amino acid data of isolated protein are currently under investigation.

ACKNOWLEDGMENT

Appreciation is extended to Drs. M. S. Zuber, Department of Agronomy, University of Missouri—Columbia, and E. B. Lillehoj, Southern Regional Research Center, USDA, New Orleans, LA, for providing the kernels and for valuable discussions regarding aflatoxin contamination in maize.

LITERATURE CITED

Adye, J.; Mateles, R. I. *Biochim. Biophys. Acta* **1964**, *86*, 418.
Brambl, R.; Gade, W. *Plant Physiol.* **1985**, *64*, 402.
Dechary, J. M.; Talluto, K. F.; Evans, W. J.; Carney, W. B.; Altschul, A. M. *Nature (London)* **1961**, *190*, 1125.
Etzlor, M. E. *Phytopathology* **1981**, *71*, 744.
Fennell, D. I.; Lillehoj, E. B.; Kwolek, W. F. *Cereal Chem.* **1975**, *52*, 314.
Hesseltine, C. W.; Shotwell, O. L.; Kwolek, W. F.; Lillehoj, E. B.; Jackson, W. K.; Bothast, R. J. *Mycologia* **1976**, *68*, 341.
Hierholzer, H. C.; Suggs, M. T. *Appl. Microbiol.* **1969**, *18*, 816.
Kommedahl, T., Williams, P. H., Eds. In *Challenging Problems in Plant Health*; American Phytopathological Society: St. Paul, MN, 1983.
Landry, J.; Moureaux, T. *Bull. Soc. Chim. Biol.* **1970**, *52*, 1021.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Marsh, S. F.; Payne, G. A. *Phytopathology* **1984**, *74*, 1284.
Mirelman, D.; Galun, E.; Sharon, N.; Lotan, R. *Nature (London)* **1975**, *256*, 414.
Newburg, D. S.; Concon, J. M. *J. Agric. Food Chem.* **1985**, *33*, 685.
Prigent, M. J.; Bourrillion, R. *Biochim. Biophys. Acta* **1976**, *420*, 112.
Roberts, W. K.; Selitrennikoff, C. P. *Biochim. Biophys. Acta* **1986**, *880*, 161.
Stoloff, L. *Proc. Am. Phytopathol. Soc.* **1976**, *3*, 156.
Stoloff, L., Scott, P. M., Eds. In *Official Methods of Analysis*, 14th ed.; AOAC: Washington, DC, 1984; pp 477–496.
Zuber; et al. *Plant Dis.* **1983**, *67*, 185.

Received for review September 18, 1986. Accepted April 6, 1987. Mention of commercial items does not imply endorsement by the U.S. Department of Agriculture.

Inhibition of Lipid Peroxidation by Ellagic Acid

Toshihiko Osawa,* Atsushi Ide, Jeng-De Su, and Mitsuo Namiki

Ellagic acid, a common plant phenol, was found to be an effective inhibitor of in vitro lipid peroxidation by the erythrocyte ghost and microsome test systems. The structure–activity relationship of ellagic acid and two of its derivatives has been carried out, and it was suggested that ellagic acid was the most potent inhibitor of the perferryl-dependent initiation step of NADPH-dependent microsomal lipid peroxidation. Ellagic acid also strongly inhibited lipid peroxidation induced by Adriamycin, but the two ellagic acid derivatives were much less effective. This difference was true of all NADPH-dependent microsomal lipid peroxidations.

Oxygen species such as hydroxy radicals, superoxide anion radicals, and singlet oxygens are proposed to be agents that attack polyunsaturated fatty acids in cell membranes and give rise to lipid peroxidation. Several reports have suggested that lipid peroxidation may lead to destabilization and disintegration of cell membranes, to liver injury and other diseases, and finally, to aging and susceptibility to cancer (Player, 1982).

Recently, much attention has focused on cellular protective systems against damages caused by oxygen radicals including enzymes, such as superoxide dismutase, GSH-peroxidase, GSH-transferase, and catalase, as well as nonenzymatic protection of polyunsaturated fatty acid by endogenous antioxidants like α-tocopherol, ascorbic acid, β-carotene, and uric acid (Ames et al., 1981). In addition, several antioxidants have been reported to play an im-

Department of Food Science & Technology, Nagoya University, Chikusa, Nagoya 464, Japan.

0021-8561/87/1435-0808$01.50/0 © 1987 American Chemical Society

portant role in prevention of carcinogenesis related to active oxygen radicals and, in some cases, to extend life span in animals (Cutler, 1984).

There are also some indications that not only endogeneous antioxidants but also dietary antioxidants may offer effective protection from peroxidative damages in living systems (Yaeger and Bjorksten, 1980). Few dietary antioxidants other than α-tocopherol have been evaluated with in vitro lipid peroxidation systems (Witting, 1980). For this reason, an intensive search for novel antioxidants from natural sources was undertaken in sesame seeds (Fukuda et al., 1985; Osawa et al., 1985a), rice hulls (Osawa et al., 1985b; Ramarathnam et al., 1986), and plant drugs (Su et al., 1986). During investigation of the strong antioxidative constituents in the *Eucalyptus* leaves, we isolated and identified β-diketone type antioxidants from *Eucalytus* leaf waxes (Osawa and Namiki, 1981, 1985). Moreover, we observed that ellagic acid is present as one of the strong antioxidative components in the alcoholic extracts of *Eucalyptus* leaves (Osawa and Namiki, 1983). Ellagic acid is widely distributed in the human diet of fruits and vegetables (Hulme, 1970) and also is a very important constituent of plant drugs (Okuda et al., 1983; Su et al., 1987). In the present study, ellagic acid was examined for the inhibition of in vitro lipid peroxidation induced by various systems, especially by Adriamycin. The antioxidative mechanism of ellagic acid is also discussed.

MATERIALS AND METHODS

Chemicals. All standard chemicals were purchased from Wako, Nakarai, Tokyo Kasei, and Sigma Chemical Co. and were of analytical reagent grade. Ellagic acid, hexahydroxydiphenic acid (HHDP), and ellagic acid tetraacetate were gifts from Sanyo-Kokusaku Pulp Co., Ltd. The purest grade biochemicals, ADP and NADPH, were obtained from Oriental Yeast Co., Ltd., and Adriamycin was a gift from Fermitalia Carlo Erba Co., Ltd.

Rabbit Erythrocyte Membrane Ghost System. Commercially available rabbit blood (50 mL) was obtained from Japan Biotest Institute Co., Ltd., and diluted with 150 mL of isotonic buffer solution (10 mM phosphate/152 mM NaCl). After centrifugation (1500*g*, 10 min), the blood was washed three times with 10 mL of isotonic buffer solution and lysed in 10 mM phosphate buffer, pH 7.4. Erythrocyte membrane ghosts were pelleted by centrifugation (20000*g*, 40 min), and the precipitate was diluted to give a suspension (2.5 mg of protein/mL) as determined by the method of Lowry et al. (1951). Peroxidation of the erythrocyte membrane ghosts induced by *tert*-butyl hydroperoxide was carried out by the method of Ames et al. (1981). Natural antioxidants were prepared by dissolving them in dimethyl sulfoxide (Me_2SO) (not more than 50 μL of Me_2SO was contained in 1-mL total volume). After incubation for 30 min, 1 mL of 2.0 M TCA/1.7 M HCl and 2 mL of 0.67% TBA/NaOH solution was added to stop the reaction. The quantity of TBA-reacting substance (TBARS) was determined at 532 nm after coloration with thiobarbituric acid (Wilbur et al., 1949).

Microsomal Lipid Peroxidation. Wister rats (8 weeks, 180–200 g) were sacrificed and their livers removed and homogenized. Microsomes were prepared by differential centrifugation by the method of Kornbrust and Mavis (1980). Fresh solutions, in 50 mM Tris-HCl buffer, were prepared each time at the concentration of 1 mg/mL microsomal protein as determined by the Lowry method. Test chemicals were dissolved in Me_2SO and added to the microsomal incubation (final concentration was less than 1%). The following additions were made and incubated at 37 °C for 30 min: 2.5 mM ADP and 0.1 mM $FeSO_4$ (ADP–Fe^{2+} system); 2.5 mM ADP, 0.1 mM EDTA, and 0.1 mM $Fe(NO_3)_3$ (ADP–Fe^{3+}–EDTA system); 5 mM NADPH in 50 mM Tris-HCl buffer solution. Adriamycin-induced lipid peroxidation was carried out by the addition of 0.1 mM Adriamycin, 1.9 mM NADP, 20 mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase (1.1 U/mL), and 9 mM $MgCl_2$, followed by incubation for 30 min in a saturated oxygen atmosphere. After incubation, formation of TBARS was measured as described above. At least eight different concentrations of antioxidants were used, and values obtained without antioxidants were taken for 100% lipid peroxidation. The value of IC_{50} means concentration of antioxidants required for 50% inhibition of microsomal lipid peroxidation.

Formation of Oxygen Radicals by the Model Systems. Hydroxy radical was generated by γ-ray irradiation (total dose 50 krad; dose rate 1.2×10^5 rad/h) under a N_2O atmosphere (Hiatt, 1971). Destruction rate of substrate (300 μM) was measured by reading the maximum UV absorption of each substrate (230–320 nm) before and after irradiation. For measurement of singlet oxygen generation, rose bengal was added to the solution of each substrate (2 mM in 25 mM phosphate buffer, pH 7.6). This solution was irradiated under oxygen atmosphere in an ice bath by a tungsten/halogen lamp (400 W) at a distance of 40 cm (Ames et al., 1981). The destruction rate of substrate was also quantified by reading maximum UV absorption of each sustrate before and after irradiation. Superoxide anion radical was generated by the method of Matsumoto and Matsuo (1977). KO_2 (0.3 mM) was added to the substrate solution (0.1 mM in 20 mL of tetrahydrofuran) in a 50-mL conical flask. The destruction rate of substrate was also measured by reading the maximum UV absorption of each substrate.

Linoleic Acid Autoxidation by Model Systems. Autoxidation of linoleic acid in the water/alcohol solution has been assayed by thiocyanate and TBA methods (Osawa and Namiki, 1985). Each sample was dissolved in chloroform or in ethanol and the resultant mixture added to a solution mixture of linoleic acid/99.0% ethanol/0.2 M phophate buffer. The mixed solution in a conical flask was incubated at 40 °C, and the peroxide value was determined at 500 nm after a color reaction with $FeCl_2$ and thiocyanate (Mitsuda et al., 1966). The formation of TBA-reacting substance was measured at 532 nm after the reaction with thiobarbituric acid (Ottolenghi, 1959).

RESULTS AND DISCUSSION

Like many other biological membranes, red blood cell membranes are prone to lipid peroxidation because of their high polyunsaturated lipid content. Initial evaluation of the antioxidative activity of typical plant phenols, including ellagic acid, was performed in the rabbit erythrocyte membrane ghost system. As shown in Figure 1, ellagic acid showed the strongest protection from lipid peroxidation induced by *tert*-butyl hydroperoxide among the plant phenols but was still less active than α-tocopherol. As suggested by Ames et al. (1981), many endogenous antioxidants such as tocopherols (vitamin E), ascorbate, glutathione, carotenoids, and uric acid play an important role for protection from oxidative damages. Plant phenols commonly present as the food components, in particular ellagic acid, may also inhibit the lipid peroxidation promoted by the hemo compounds associated with the phospholipids of biological membrane in blood (Chiu et al., 1982).

Numerous enzymatic lipid peroxidation systems have reported that addition of iron or iron–chelate complex is required for promotion of peroxidation (Aust and Svingen,

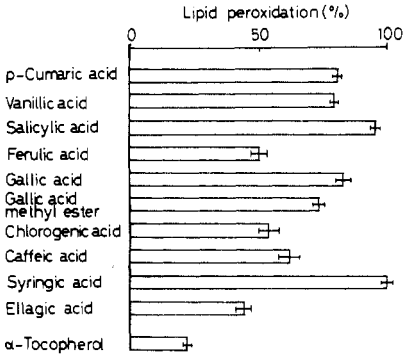

Figure 1. Antioxidative assay of plant polyphenols by erythrocyte membrane ghost system. Antioxidants of 100 μM were used for this experiment, and details are shown in Materials and Methods. The values obtained without antioxidants were taken for 100% lipid peroxidation. Error bars denote standard deviation (n = 3).

Table I. Inhibition of Lipid Peroxidation in the Rat Liver Microsome System by Polyphenols

compound	IC_{50}[a] ADP-Fe^{2+}/NADPH	IC_{50}[a] ADP-Fe^{3+}/EDTA-Fe^{3+}/NADPH
ellagic acid	20 ± 1	23 ± 2
p-coumaric acid	330 ± 8	>100
vanilic acid	400 ± 6	>100
salycilic acid	460 ± 5	>100
ferulic acid	>500	>100
gallic acid	>500	8.0 ± 0.3
gallic acid methyl ester	>500	>100
chlorogenic acid	>500	67 ± 4
caffeic acid	340 ± 8	80 ± 6
syringic acid	>500	>100
α-tocopherol	320 ± 3	0.80 ± 0.02
propyl gallate	>500	20 ± 3

[a] IC_{50} = concentration (μM) for 50% inhibition of lipid peroxidation in model systems. Details shown in Materials and Methods. Reported values are mean ± SD (n = 3).

1982). This requirement has been well studied in NADPH-dependent microsomal lipid peroxidation, especially by addition of ADP-Fe^{2+} or by ADP-Fe^{3+}/EDTA-Fe^{3+} (Hochstein and Ernster, 1963). Enhancement of lipid peroxidation by ADP-Fe^{2+} complex and NADPH has been considered to have a correlation with the perferryl ion dependent initiation step (Ernster and Nordenbrand, 1967; Pederson and Aust, 1972, 1975); addition of EDTA has been suggested to promote the hydroperoxide-dependent initiation step of microsomal lipid peroxidation (Pederson and Aust, 1972, 1975; Lai and Piette, 1978). Almost all plant phenols tested can inhibit microsomal lipid peroxidation (Table I). Among them, ellagic acid inhibited lipid peroxidation induced by both systems, in particular the perferryl ion dependent initiation step of lipid peroxidation. α-Tocopherol inhibited the lipid peroxidation promoted by the hydroperoxide-dependent initiation step (ADP-Fe^{3+}/EDTA-Fe^{3+}/NADPH), and propyl gallate showed almost the same inhibitory effect as ellagic acid against lipid peroxidation induced by ADP-Fe^{3+}/EDTA-Fe^{3+}/NADPH system but showed only a weak antioxidative activity in the ADP-Fe^{2+}/NADPH system.

To confirm the structure–activity relationship, the in vitro antioxidative activities of ellagic acid and two of its derivatives were compared. It is essential to note that Adriamycin, a member of the anthracycline group of antineoplastic agents widely used in the medical treatment of carcinoma, has been reported to cause several side effects. Cardiotoxicity, one of the severe side effects, has been reported to be caused by lipid peroxidation induced

Table II. Inhibition of Lipid Peroxidation in the Rat Liver Microsome System by Ellagic Acid and Two Derivatives

compound	IC_{50}[a] ADP-Fe^{2+}/NADPH	IC_{50}[a] ADP-Fe^{3+}/EDTA-Fe^{3+}/NADPH	IC_{50}[a] Adriamycin
α-tocopherol	>100	0.80 ± 0.02	5.5 ± 0.2
propyl gallate	>100	20 ± 3	0.30 ± 0.02
ellagic acid	20 ± 1	23 ± 2	0.10 ± 0.01
hexahydroxydiphenic acid	>100	>100	7.0 ± 0.5
ellagic acid tetraacetate	>100	>100	31 ± 3

[a] IC_{50} = concentration (μM) for 50% inhibition of lipid peroxidation in model systems. Reported values are mean ± SD (n = 3).

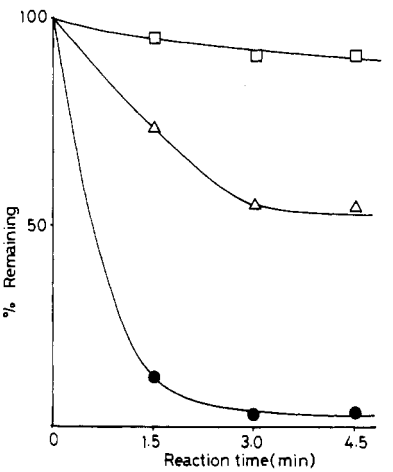

Figure 2. Decomposition of antioxidants by superoxide anion radicals generated in the KO_2/THF system. Remaining percentages of α-tocopherol (●), propyl gallate (△), and ellagic acid (□) were calculated by reading the maximum UV absorption during the reaction at definite invervals. Results are averages of three replicates.

by oxygen radicals (superoxide anion radical, hydroxy radical, singlet oxygen) derived from the quinone structure of anthracycline antibiotics; the detailed mechanisms are still unknown (Mimnaugh et al., 1983). If antioxidants inhibit lipid peroxidation induced by Adriamycin, they could be of value in combination with chemotherapetic agents such as Adriamycin. In fact, ellagic acid inhibited strongly the lipid peroxidation induced by Adriamycin, but the two ellagic acid derivatives were much less active (Table II). Blocking the phenolic hydroxy group reduced the antioxidative activity although *o*-dihydroxy group of ellagic acid was speculated to be regenerated via enzymatic hydrolysis (Euranto, 1969). Moreover, some lipophilic property caused by lactone linkage in ellagic acid seems to be very important for antioxidative activity, and this result may be attributable to better subcellular distribution relative to HHDP. The mode of action of ellagic acid on the enzymatic generation and control of oxygen radicals is not clear, but ellagic acid was found to have strong inhibitory power against radical-induced oxidative destruction of membrane lipids in both erythrocyte and microsomal systems. Using the model systems, we examined the possibility whether ellagic acid may scavenge superoxide anion radical, singlet oxygen, and hydroxy radical.

As shown in Figure 2, sensitivity of ellagic acid to superoxide anion radical has been evaluated; however, only 9% of ellagic acid was destroyed by superoxide anion radical after 4.5 h of incubation in KO_2/tetrahydrofuran solution compared to 96% of α-tocopherol and 46% of propyl gallate. Ellagic acid was also less affected by both hydroxy radical and singlet oxygen (Figures 3 and 4).

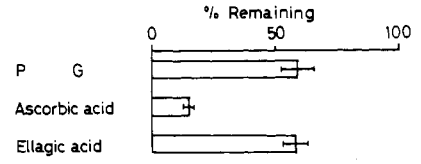

Figure 3. Remaining percentages of propyl gallate (PG), ascorbic acid, and ellagic acid shown after γ-irradiation. Error bars denote standard deviation (n = 3).

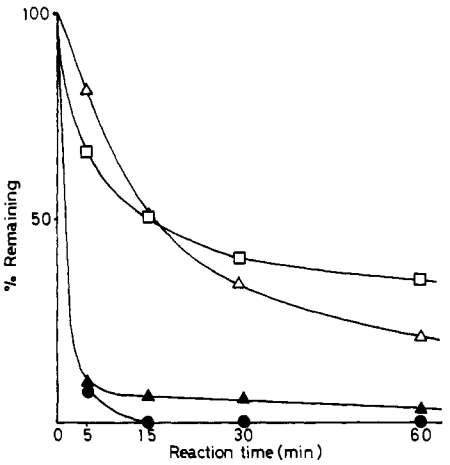

Figure 4. Destruction of antioxidants by singlet oxygen: α-tocopherol (●); ascorbic acid (▲); propyl gallate (△); ellagic acid (□). Results are averages of three replicates.

Although 85% of ascorbic acid was destroyed by hydroxy radical, 57% of ellagic acid and 59% of propyl gallate still remained after γ-irradiation. After 15-min incubation almost 100% of α-tocopherol and 90% of ascorbic acid were destroyed by singlet oxygen but only 50% of ellagic acid and propyl gallate act as the scavengers of singlet oxygen (Figure 4). These results showed that strong inhibitory effect of ellagic acid against lipid peroxidation induced by Adriamycin cannot be attributed to its oxygen radical scavenging ability because α-tocopherol scavenged strongly both superoxide anion and hydroxy radicals but exhibited only 1/50th the antioxidative activity of ellagic acid although our evaluation has been carried out only by simple model systems.

The inhibitory effect of ellagic acid against autoxidation of linoleic acid was also examined in the water/alcohol model systems with the thiocyanate and TBA methods. Ellagic acid had a marked inhibition of hydroperoxide formation of linoleic acid, and it was as effective as standard antioxidants such as propyl gallate and α-tocopherol (Figure 5). It was also shown that ellagic acid inhibited the production of TBA-reacting substances as strong as propyl gallate. Strong inhibitory activity of nonenzymatic lipid peroxidation in the water/alcohol system by ellagic acid suggested the potential utilization of ellagic acid as an effective antioxidant for food systems.

Recent studies have shown that ellagic acid inhibits the mutagenicity of diol epoxides of several polycyclic aromatic hydrocarbones (PAH) in the Ames test using *Salmonella typhimurium* (Wood et al., 1982), epidermal metabolism and DNA-binding of benzo[*a*]pyrene (Del Tito et al., 1983), and PAH-induced skin carcinogenesis in Balb/C mice (Mukhtar et al., 1984). Although our data were obtained by enzymatic and nonenzymatic model systems, dietary antioxidants, especially ellagic acid, may have an important role for protection of many diseases related to lipid peroxidation including aging and carcinogenesis.

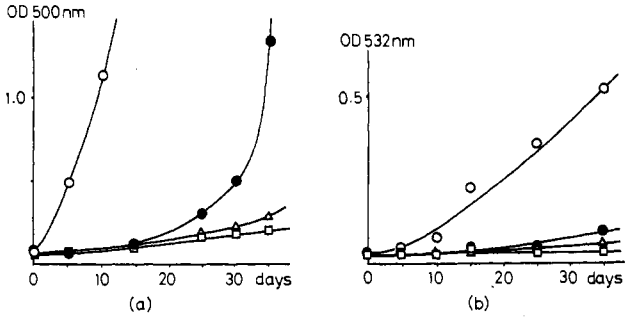

Figure 5. Antioxidative activity of ellagic acid and standard antioxidants: control (○); ellagic acid (□); α-tocopherol (●); propyl gallate (△). Key: (a) thiocyanate method; (b) thiobarbituric acid test (TBA test). Results are averages of three replicates.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

Registry No. HHDP, 32773-02-7; NADPH, 53-57-6; ellagic acid, 476-66-4; ellagic acid tetraacetate, 4274-26-4; Adriamycin, 23214-92-8.

LITERATURE CITED

Ames, B. N.; Cahcart, R.; Schwiers, E.; Hochstein, P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6858.
Aust, S. D.; Svingen, B. A. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic: New York, 1982; Vol. V, p 1.
Chiu, D.; Lubin, B.; Shohet, S. B. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic: New York, 1982; Vol. V, p 115.
Cutler, R. G. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic: New York, 1984; Vol. VI, p 371.
Del Tito, B. J., Jr.; Mukhtar, H.; Bickers, D. R. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 388.
Ernster, L.; Nordenbrand, K. *Methods Enzymol.* **1967**, *10*, 574.
Euranto, E. K. In *The Chemistry of Carboxylic Acids and Esters*; Patai, S., Ed.; Interscience: London, 1969; p 505.
Fukuda, Y.; Osawa, T.; Namiki, M.; Ozaki, T. *Agric. Biol. Chem.* **1985**, *49*, 301.
Hiatt, R. In *Organic Peroxides*; Swern, D., Ed.; Wiley-Interscience: New York, 1971; Vol. II, p 1.
Hochstein, P.; Ernster, L. *Biochem. Biophys. Res. Commun.* **1963**, *12*, 388.
Hulme, A. C. In *The Biochemistry of Fruits and Their Products*; Academic: London, 1970; Vol. I.
Kornbrust, D. J.; Mavis, R. D. *Lipid* **1980**, *15*, 315.
Lai, C.; Piette, L. H. *Arch. Biochem. Biophys.* **1978**, *190*, 27.
Lowry, O. H.; Rosenbrough, N. J.; Forr, A. L.; Randall, T. J. *J. Biol. Chem.* **1951**, *193*, 265.
Matsumoto, S.; Matsuo, M. *Tetrahedron Lett.* **1977**, *18*, 1999.
Mimnaugh, E. G.; Gram, T. E.; Trush, M. A. *J. Pharm. Exp. Ther.* **1983**, *226*, 806.
Mitsuda, H.; Yasumoyo, K.; Iwai, K. *Eiyo to Shokuryo* **1966**, *19*, 210.
Mukhtar, H.; Das, M.; Del Tito, B. J., Jr.; Bickers, D. R. *Biochem. Biophys. Res. Commun.* **1984**, *119*, 751.
Okuda, T.; Kimura, Y.; Yoshida, T.; Hatano, T.; Okuda, H.; Arichi, S. *Chem. Pham. Bull.* **1983**, *31*, 1625.
Osawa, T.; Namiki, M. *Agric. Biol. Chem.* **1981**, *45*, 735.
Osawa, T.; Namiki, M. In *Research in Food Science and Nutrition*; McLoughlin, J. V., McKenna, B. M., Eds.; Boole: Dublin, 1983; Vol. 3, p 49.
Osawa, T.; Namiki, M. *J. Agric. Food Chem.* **1985**, *33*, 77.
Osawa, T.; Nagata, M.; Namiki, M.; Fukuda, Y. *Agric. Biol. Chem.* **1985a**, *49*, 3351.
Osawa, T.; Ramarathnam, N.; Kawakishi, S.; Namiki, M.; Tashiro, T. Agric. *Biol. Chem.* **1985b**, *49*, 3085.
Ottolenghi, A. *Arch. Biochem. Biophys.* **1959**, *79*, 355.
Pederson, T. C.; Aust, S. D. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 789.
Pederson, T. C.; Aust, S. D. *Biochem. Biophys. Acta* **1975**, *385*, 232.

Player, T. J. In *Free Radicals, Lipid Peroxidation and Cancer*; McBrien, D. C. H., Slater, T. F., Eds.; Academic: London, 1982; p 173.
Ramarathnam, N.; Osawa, T.; Namiki, M.; Tashiro, T. *J. Sci. Food Agric.* **1986**, *37*, 719.
Su, J.-D.; Osawa, T.; Namiki, M. *Agric. Biol. Chem.* **1986**, *50*, 199.
Su, J.-D.; Osawa, T.; Kawakishi, S.; Namiki, M. *Agric. Biol. Chem.* **1987**, in press.
Wilbur, K. M.; Bernheim, F.; Shapiro, O. W. *Arch. Biochem.* **1949**, *24*, 305.
Witting, L. A. *Free Radicals in Biology*; Pryor, W. A. Ed.; Academic: New York, 1980; Vol. IV, p 295.
Wood, A. W.; Wang, H. T.; Chang, R. L.; Newmark, H. L.; Lehr, R. E.; Yagi, H.; Sayer, J. M.; Jerina, D. M.; Conney, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5513.
Yaeger, L. L.; Bjorksten, J. *In Autoxidation in Food and Biological Systems*; Simic, M. G., Karel, M., Eds.; Plenum: New York, 1980; p 405.

Received for review May 6, 1986. Revised manuscript received April 23, 1987. Accepted June 13, 1987.

Environmental Impact of Agricultural Nitrogen and Phosphorus Use

Andrew N. Sharpley,* S. J. Smith, and J. W. Naney

The impact of fertilizer application on the amount of nitrogen (N) and phosphorus (P) in surface runoff from 20 watersheds and ground water from 45 shallow wells (<20-m water tables) has been assessed for the past several years on agricultural land in the Southern Plains. The major proportion of N and P transported in surface runoff was in the particulate form (averaging 64 and 75%, respectively). Soluble and total P concentrations from unfertilized and fertilized watersheds were consistently greater than the critical levels associated with accelerated eutrophication (10 and 20 $\mu g\ L^{-1}$, respectively). In most cases, nitrate- and ammonium-N concentrations in ground water were within acceptable limits for potable use (10 mg L^{-1} NO_3-N) and for fish (2.5 mg L^{-1} NH_4-N). In general, therefore, these agricultural practices had little environmental impact on ground water quality, although P enrichment of surface runoff may stimulate accelerated eutrophication of small lakes and impoundments in the Southern Plains. Measures to reduce the environmental impact of agricultural N and P use are discussed.

The transport of nitrogen (N) and phosphorus (P) in surface runoff from agricultural land, increased by fertilizer applications, often controls the biological productivity of surface waters (Loehr, 1974; Schindler, 1977; Vollenweider and Kerekes, 1982). Although both N and P have been associated with accelerated eutrophication, most attention has focused on P. This results from the fact that exchange of N between the atmosphere and a waterbody and fixation of atmospheric N by some blue-green algae, can be sufficient to prevent N from limiting biological productivity. Thus, for most lakes, P is often the limiting element and its control is of prime importance in reducing the accelerated eutrophication of a water body. As a result, Sawyer (1947) and Vollenweider (1971) proposed critical soluble (SP) and total P (TP) concentrations of 10 and 20 $\mu g\ L^{-1}$ respectively, which if exceeded may accelerate the eutrophication of lakes and impoundments.

Due to the rapid fixation of fertilizer P by surface soil material, ground water contamination by fertilizer material is often associated with nitrate-N (NO_3-N). This results from the fact that N fertilizer application to agricultural land exceeds that of the other mineral elements (Hargett and Berry, 1985). In the ammonium-N (NH_4-N) form, N is fairly immobile in soil. However, under most conditions NH_4-N is converted to NO_3-N, which can readily move with soil water. Nitrate-N not utilized by the crop, incorporated into soil organic matter, or denitrified, can leach from the soil profile into the ground water where it has the potential to pose an environmental hazard. Acceptable limits of NO_3-N concentration of 10 and 100 mg L^{-1} have been established for human and livestock consumption, respectively, and NH_4-N concentrations above 0.5 and 2.5 mg L^{-1} may be harmful to humans and fish, respectively (U.S. EPA, 1973).

The nonpoint pollution of lakes and impoundments via the contamination of surface and ground water with fertilizer N and P is now recognized as one of the nation's major water quality problems. For example, in 1984, the EPA reported to Congress that 6 of 10 EPA regions found nonpoint sources to be the principal remaining cause of water quality problems and that virtually every state reported some kind of problem related to these sources (U.S. EPA, 1984). More specifically, the southwestern United States, a large and important farming and ranching area, is undergoing greater rural and urban demands on its water supplies as the population increases. As a result, there has been a renewed urgency in obtaining detailed information on surface and ground water supplies associated with current and proposed agricultural management practices. Although several studies have documented the effect of N and P fertilizer applications on surface and ground water quality in the corn belt region of the midwest United States (Burwell et al., 1975, 1977; Hanway and Laflen, 1974; Schuman et al., 1973a,b), little information is available for the southwest, especially Oklahoma and Texas.

This paper presents the results of an 8-year monitoring program of 20 watersheds and 45 shallow wells (<20-m water table depth) in Oklahoma and Texas, to assess the impact of agricultural practices on N and P levels in surface and ground water quality in this area. In some cases, wells were located on watersheds not monitored for surface runoff quantity and quality.

Water Quality and Watershed Research Laboratory, USDA—ARS, Durant, Oklahoma 74702-1430.

This article not subject to U.S. Copyright. Published 1987 by the American Chemical Society