dependent upon the experimental culture conditions. Variations in experimental culture conditions resulted in the formation of TCAB or the triazene instead of or in addition to the azoxybenzene.

At present the environmental significance of the azoxybenzene is not known. The chemical and physical characteristics of the azoxybenzene are such that if it were present in aniline-based pesticide-treated soils, it would be detected by the same technique used for monitoring TCAB formation. A cursory examination of all of our experimental data previously published (Kaufman *et al.*, 1971; Kearney *et al.*, 1969, 1970; Plimmer and Kearney, 1969; Plimmer *et al.*, 1970; Chisaka and Kearney, 1970) indicates that azoxybenzene type compounds were not present even in soils treated with exceptionally high rates of 3,4-DCA- or 3,4-DCA-containing pesticides.

Plimmer and Kearney (1969) detected the formation of 3,3′,4,4′-tetrachloroazoxybenzene during the photolysis of 3,4-DCA. Their system, however, involved irradiation by light wavelength >280 nm in benzene solution with benzophenone as a sensitizer in the presence of oxygen. They were unable to detect the azoxybenzene in soils under more natural conditions. Nevertheless, the chemical and physical behavior of azoxybenzenes in the environment will be experimentally assessed. Note: While this manuscript was in its final preparation the authors learned of a recent report that 3,4-dichlorophenylhydroxylamine was formed from 3,4-dichloroaniline by the action of peroxidase (Bordeleau *et al.*, 1972).

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Effect of Gallic (3,4,5-Trihydroxybenzoic) Acid on Iron Availability

Gallic acid added at 2% to experimental rat diets increased hemoglobin and hematocrit repletion of anemic rats fed ferrous sulfate or ferric citrate. This measure of biological availability of iron was more pronounced in the poorer utilized ferric salt than the better absorbed and utilized ferrous sulfate. Concord grape juice, known to contain free gallic acid and compounds containing galloyl groups, did not have a similar effect on the availability of iron from ferric citrate.

Gallic acid is present in a large variety of edible plants either in free form or as part of naturally occurring phenolic compounds, *i.e.*, anthocyanins, catechins, leucoanthocyanidins, gallotannins. (For occurrence of these compounds, see Hegnauer, 1962–1969). Metabolism studies in rats and rabbits ingesting gallic acid have shown that 4-*O*-methylgallic acid is the major metabolite in the urine. Decarboxylation accounted for a second metabolite identified as pyrogallol (Booth *et al.*, 1959). Gallic acid appears to increase the requirement for dietary methyl donors such as choline and methionine (Booth *et al.*, 1961).

Propyl gallate, a synthetic antioxidant widely used in fats, oils, and other food products (National Academy of Sciences, 1965), was shown to lower slightly hemoglobin levels in rats when fed at high dietary levels (Orten *et al.*, 1948). This suggests a chelation effect that interferes with the absorption of iron (Kuhn *et al.*, 1968). In contrast, however, Concord grape juice, which contains both free gallic acid and galloyl groups in the B-ring of its major anthocyanin, delphinidin 3-monoglucoside (Mattick *et al.*, 1967), enhanced iron availability and increased hemoglobin concentration in children (Fishbein *et al.*, 1938).

The objective of this study was to investigate the availability of iron from two iron salts by hemoglobin and hematocrit repletion in young anemic rats when gallic acid is incorporated into experimental diets.

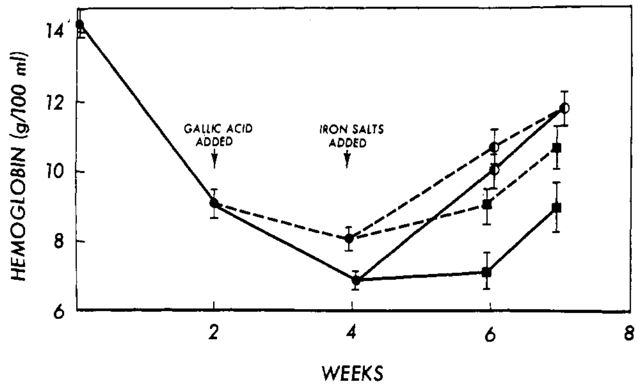

Figure 1. Blood hemoglobin changes in rats fed diets containing recrystallized gallic acid and supplemented with either ferric citrate or ferrous sulfate. ●—●, 0 gallic acid; ■—■, 0 gallic acid + ferric citrate; ◐—◐, 0 gallic acid + ferrous sulfate; ●- - -●, 2% gallic acid; ■- - -■, 2% gallic acid + ferric citrate; ◐- - -◐, 2% gallic acid + ferrous sulfate

METHODS AND MATERIALS

Sixty Sprague-Dawley weanling male rats, housed in individual cages, were fed the following basal diet for 2 weeks (in percent): casein, 25; corn oil, 10; cellulose, 4; dextrose, 18.2; dextrin, 18.2; sucrose, 18.2; choline chloride (50%), 0.4; vitamins, 1; salts, 5.0 without iron. After 2 weeks, 30 rats were fed this diet containing 2% gallic acid, recrystallized twice, substituted by weight with the sucrose. The other 30 rats continued on the basal diet. After another 2-week period, the animals fed either the gallic acid or basal diet were segregated into two groups of 15 rats per group and ferric citrate or ferrous sulfate was added to both diets. These four dietary treatments were continued for 3 additional weeks. The amount of iron added from both salts as determined by atomic absorption spectrophotometry was 15 mg/kg.

Blood was taken from all rats at 0, 2, 4, 6, and 7 weeks and hemoglobin and hematocrits were determined by the methods described in Seiverd (1968). Hemoglobin repletion of the anemic rats when the iron salts were added to the diet at 4 weeks has been described as a measure of biological availability of iron (Pla and Fritz, 1970). Statistical procedures were accomplished by techniques described in Steel and Torrie (1960).

RESULTS AND DISCUSSION

The effect of gallic acid on hemoglobin repletion in rats fed the two iron salts is presented in Figure 1. After the initial 2 weeks, the rats fed diets containing no iron supplementation exhibited a depression of hemoglobin concentration of about 36%. When gallic acid was added to the diet and blood was analyzed for hemoglobin at 4 weeks, the rats had a significantly ($p < 0.05$) higher hemoglobin concentration as compared to the group fed the basal diet, although it was further depleted from the 2-week value. After the iron salts were added to the diets, the rats consuming ferrous sulfate had significantly ($p < 0.05$) higher levels of hemoglobin than the rats fed the ferric citrate. This reflects the relatively higher biological value of the iron in ferrous sulfate (Fritz *et al.*, 1970).

A comparison of the mean hemoglobin values at both 6 and 7 weeks (Figure 1) shows that the rats consuming the diet containing ferric citrate without gallic acid had a significantly ($p < 0.05$) lower value than the animals fed the diet with ferric citrate and gallic acid. The rats fed the ferrous sulfate diets had statistically similar hemoglobin values regardless of additional gallic acid. The hematocrit values correlated with the hemoglobin levels of all treatments. Thus, the less utilized ferric salt may increase its availability of iron under the influence of gallic acid acting as a chelating or sequestering agent enhancing iron absorption.

A variety of food constituents, including dietary protein (Coons, 1964), ascorbic acid (Brise and Hallberg, 1962), and vitamin E (Greenberg *et al.*, 1957) have been reported to improve the absorption and utilization of iron, although some of the findings with the vitamins apparently are controversial (Chaney and Barnhart, 1964).

Another 7-week hemoglobin repletion test in rats drinking Concord grape juice and fed diets containing ferric citrate showed no improvement in iron availablity, as compared to water drinking, or depigmented juice drinking controls.

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