

the same reaction patterns as the antisera absorbed with human RBC and human WBC. Absorption of the two CLL antisera with cells from a CLL patient removed the cytotoxic activity for cells from all CLL and ALL patients tested.

The AML and CGL antisera after absorption with cells from a CLL patient still reacted with certain CGL and AML donors. The pattern of reactivity was the same as after absorption with human RBC and WBC. Absorption of the AML antiserum with cells from a CGL donor removed the reactivity for the absorbing cell and for cells of certain individual AML and CGL donors but left good reactivity for others. Absorption of the AML antiserum with cells from an AML patient removed the reactivity for cells from all AML donors but left activity for cells from some CGL patients.

Absorption of the CGL antiserum with cells from an AML donor removed the reactivity for cells from all AML patients, but left reactivity for cells from some CGL patients. Absorption of the same antisera with cells from a patient with CGL removed the reactivity for cells of all CGL and AML patients tested. When the antisera to AML and CGL were absorbed with cells from different individual AML and CGL donors, different patterns of reactivity could be noted when these antisera were tested with cells from other AML and CGL patients. Activity was always removed for the absorbing cell.

The antisera to cells from two different CLL donors appear to be detecting a single antigen common to all ALL and CLL patients although absorptions with cells from different individual CLL and ALL patients must be performed. It appears, however, that the AML and CGL antisera are detecting more than one antigen and that some AML or CGL patients lack all of the antigens being detected by these antisera, or that antigens are expressed on these cells in a way that is refractory to agglutination and lysis. Absorption of monkey antisera 3 and 4 with cells from additional CGL and AML patients will help to resolve the number of different antigen-antibody systems being detected by these reagents. Production of additional antisera to cells from the nonreactive AML and CGL patients will also help to define additional specificities.

The four antisera after absorption with peripheral blood leukocytes failed to react by all three methods with

thymus cells from five different donors and additional absorption of the antisera with 10^9 thymus cells per milliliter from one donor failed to alter the reactivity with leukemia cells. Suspension cultures of human lymphocytes derived from normal peripheral blood leukocytes from two different donors (6) also failed to react with the absorbed antisera.

When reactive CLL, AML, or CGL cells are treated with trypsin (0.25 percent) or neuraminidase (0.2 unit/ 10^6 cells), the cells no longer react with their respective antisera. Leukemic cells are still more than 90 percent viable after either enzymic treatment. Normal peripheral blood lymphocytes treated with trypsin or neuraminidase fail to react with the antisera to leukemic cells. CLL cells (1.5×10^8) were incubated in 0.25 percent trypsin for 20 minutes at 37°C. Soya bean trypsin inhibitor was then added, and 12 mg of the digest was fractionated on Sephadex G-150 with 0.15M phosphate-buffered saline. Leukemic antigen activity was determined by inhibition of cytotoxicity. All antigen activity was recovered in the first protein peak, which was excluded from Sephadex G-150 (Fig. 1A). This peak was not seen in the trypsin-trypsin inhibitor material that was incubated and fractionated as a control (Fig. 1B).

The results indicate that primate antisera to cells from individual leukemic donors can detect antigens that distinguish CLL or ALL cells from

AML or CGL cells and thus appear to be leukemia type-specific. There seems to be a common antigen on ALL and CLL cells detectable by the antisera to CLL. The antisera to AML and CGL are detecting more than one antigen. The antigen (or antigens) detected on the CLL, AML, and CGL cells is lost by digestion with trypsin or neuraminidase, and tryptic digests of CLL cells contain antigen that has a high molecular weight. The antisera may be important reagents for the diagnosis and immunotherapy of leukemia.

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6. Human lymphocyte suspension cultures RPMI 4098 and 6237 were obtained from Associated Biomedical Systems, Inc., Buffalo, N.Y. Both of these cell lines are reported to be free of Epstein Barr virus.
7. Supported by NIH grant CA 08975 to Duke University and by grant FR 00165 to Yerkes Primate Research Center.

17 July 1972

Sweet Taste of Water Induced by Artichoke (*Cynara scolymus*)

Abstract. *Exposure of the tongue to artichoke can make water taste sweet. Two major active components of artichoke are the salts of chlorogenic acid and cynarin. The sweetening of substances by temporarily modifying the tongue, rather than by adding a substance sweet in itself, may provide an alternative to currently used nonnutritive sweeteners.*

The artichoke (*Cynara scolymus*), known to man since 700 B.C. (1), was originally popular partly because of the belief that it could be used as a diuretic and as an aphrodisiac (2); however, no unusual effects on the sense of taste were noted (3). To the best of our knowledge, the earliest published report of a taste-modifying property of the artichoke was Blakeslee's account of the 1934 AAAS biologists' dinner. After eating globe artichokes as the salad course, 60 percent of the nearly 250 people present reported that water tasted different—in most cases, it tasted sweet (4). Others have also noted

anecdotally that beverages such as milk and wine, as well as water, taste sweet after the subjects have eaten artichokes, but this effect has not been studied systematically, and the artichoke constituents responsible have not been identified (5).

In initial tests, we compared the effects of artichoke on water with its effects on solutions of different taste qualities (that is, sucrose, citric acid, quinine hydrochloride, and sodium chloride). All solutions were sweetened to some degree by the artichoke, a suggestion that the common solvent, water, was primarily affected (6). We quanti-

fied the sweetening effects of artichoke by comparing the sweetness of water, after administration of either artichoke extract (7) or two artichoke constituents, to the sweetness of sucrose. Judgments of sweetness were obtained from six subjects with Stevens' method of magnitude estimation as modified by Smith and McBurney (8). Subjects were instructed to break the total intensity of each stimulus into the intensities for each perceived quality using any numbers that seemed appropriate; however, subjects usually gave only "sweet" responses. All stimulus solutions were warmed to 34°C and were delivered to the extended tongue through a McBurney flow system (9) at the rate of 4 ml/sec. Each sucrose stimulus was preceded by a 20-second water rinse. Since artichoke extract and the two constituents were not available in sufficient quantities to permit delivery through the flow system, subjects held 10 ml of the appropriate solution in their mouths for 2 minutes (10), then spit it out, and placed their tongues in the water delivered by the flow system. The 2-minute exposure to artichoke extract and constituents was repeated before each water stimulus. The water was free of organic compounds, was as near neutral pH as possible, and had a resistance in excess of 18 megohms.

Subjects judged each sucrose concentration twice, and judged water after each artichoke concentration once. The sweet taste of water can be intense. For example, exposure of the tongue to the extract from one-fourth of an artichoke heart (that is, 10 ml of a 2.75 percent solution of artichoke extract) makes water taste as sweet as 2 teaspoons of sucrose in 6 ounces of water (Fig. 1). The artichoke-induced sweetness is similar to another phenomenon, where the taste of water varies with the substance that precedes it on the tongue. For example, water tastes bitter-sour after exposure to NaCl (11), bitter after exposure to sucrose, salty after exposure to urea, and sweet after exposure to acids, quinine, caffeine, and potassium chlorate (12-14). Responses to water in the taste nerves of several animal species have also been shown to depend on the nature of the substance preceding the water (15). The major difference between the artichoke-induced sweet taste of water and other sweet tastes of water is in the duration of the effect. The artichoke-induced sweetness lasts longer than most of the other induced sweetnesses (16).

Chlorogenic acid (3-caffeoylquinic acid) and cynarin (1,5-dicaffeoylquinic

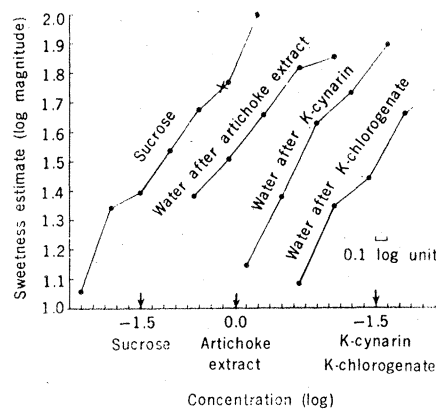


Fig. 1. The magnitude estimates of the sweetness of sucrose, and the sweetness of water after exposure of the tongue to extract and to potassium salts of cynarin (K-cynarin) and chlorogenic acid (K-chlorogenate). The abscissa shows the concentration of sucrose when it was the stimulus solution, and the inducing concentration of artichoke extract or constituents when water was the stimulus solution. The amount of artichoke extract is expressed as the log of the percent powder by weight (7). The sucrose and the potassium salts of cynarin and chlorogenic acid are expressed as the log of the molar concentration. The X on the sucrose function indicates the sweetness of 2 teaspoons of sucrose in 6-ounces of water (0.16M). The plots of sucrose and of water after artichoke extract are arbitrarily placed on the abscissa. For example, -1.7 log molar sucrose is located 2 units to the left of the sucrose arrow on the abscissa.

acid) were isolated from the artichoke extract by chromatography on Sephadex, and were identified from their ultraviolet absorption spectra and from their characteristic chromatographic mobility. The potassium salts of these acids were used in psychophysical tests, as potassium is the predominant cation in artichoke (17). The two acids, as well as their isomers, have been identified previously in artichoke (18). The sweetness of water after exposure of the tongue to potassium salts of cynarin and chlorogenic acid, and the sweetness of water after exposure of the tongue to artichoke extract are similar (Fig. 1). The duration of the sweet taste is also similar for the three substances. The chlorogenic acid concentration in our highest concentration of artichoke extract was .014M—this accounts for 29 percent of the sweetness induced by the highest artichoke extract concentration (Fig. 1). The exact concentration of cynarin in our artichoke extract is not known, but is probably not sufficient to account for the remaining sweetness produced by the artichoke extract. Thus, although cynarin and chlorogenic acid are major sources of

the sweetness, they are not the only sources. It is possible that the other isomers of these substances also possess similar taste properties.

We have evaluated the sweetness of water after exposure of the tongue to artichoke in a total of 40 subjects, 27 males and 13 females (only six of these subjects are included in Fig. 1, as only these six were tested with the potassium salts of cynarin and chlorogenic acid, as well as with artichoke extract). The data from six male subjects who failed to observe any sweetness were omitted in Fig. 1. Whether or not the insensitivity to the effect has a genetic basis, as does the insensitivity of certain persons to PTC (phenylthio-carbamide) and related compounds (19), is unknown.

Sweetness can be induced by exposing the tongue to another plant substance, miracle fruit (*Synsepalum dulcificum*). However, with miracle fruit, sour substances rather than water are sweetened, the sweetness lasts much longer than that produced by artichokes, and fewer individuals fail to observe the effect (20). To the best of our knowledge, the artichoke is the only plant known to have taste modifying properties, which is also a commonly consumed food in the United States (21).

The sweetness induced by artichokes is not produced by mixing a sweet-tasting additive with the substance to be tasted, but rather by temporarily altering the tongue so that a normally nonsweet substance tastes sweet itself. This has potential implications for alternatives to currently available non-nutritive sweeteners, and there is the possibility that research on taste modification may contribute to the improved palatability of food derived from sources such as algae or yeast, which may be needed increasingly in the future (22).

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 6. The failure of acid to be selectively sweetened is an indication that the mechanism of sweetness induction by artichoke cannot be the same as that of sweetness induction by miracle fruit.
 7. The artichoke extract was prepared from commercially available frozen globe artichoke hearts. These were boiled for 8 minutes, were cooled, and were then blended with ethanol (final concentration, 50 percent) for 5 minutes at high speed. The suspension was centrifuged at 10,000g for 30 minutes, the ethanol was evaporated, and the remaining aqueous extract was freeze-dried for storage [yield, 36 to 44 g of powder from 1000 g of raw artichoke hearts (about 40 hearts)]. The concentrations of artichoke extract in this experiment are given as the percent powder by weight.
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 10. The sweetening effect is apparent after even 15 seconds of exposure to artichoke, and the effect is maximal at approximately 1 minute. The tastes of the artichoke extract and constituents were not themselves evaluated with the flow system because of the limited quantities available. However, our subjects described the tastes as complex, and identified both bitter and sour components.
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- 27 October 1972

Beggiatoa: Occurrence in the Rice Rhizosphere

Abstract. *A catalase-like activity surrounding the root tips of rice plants favors the presence of Beggiatoa, an organism capable of oxidizing the hydrogen sulfide which is toxic to rice and is found in paddy soil conditions. Beggiatoa has now been isolated from rice field soil. A mutually favorable interaction between rice and this bacterium is suggested.*

Anaerobic organisms such as *Desulfovibrio* spp. abound in submersed soils and produce large quantities of hydrogen sulfide by the reduction of sulfate (1). Theoretical predictions of toxic concentrations of H_2S in rice paddies in Louisiana and Texas (at least 0.1 part per million of H_2S in soils containing abundant iron) (2) have been confirmed by field measurements of S^{2-} concentrations with a sulfide electrode (3). These minimal concentrations have been shown to inhibit significantly the activities of cytochrome oxidase and other oxidase enzymes in rice seedling homogenates (4).

The occurrence of such concentrations of H_2S during the heading-flowering stage of rice plant development in areas of single harvest yields of nearly 6000 pounds of rough rice per acre (5500 kg/ha) led us to postulate detoxification of H_2S by a biological system. *Beggiatoa*, a filamentous bacterium capable of oxidizing H_2S to sulfur intracellularly, was isolated from paddy soil in the Crowley, Louisiana, area and its physiological characteristics were verified. This bacterium is autointoxicated by hydrogen peroxide and the compound must be decomposed externally for optimum growth.

We studied rice plants growing in a greenhouse, a growth chamber, and gnotobiotic nutrient solutions. Using the polarographic method of Rodriguez-

Kabana and Truelove (5) for the detection of catalase (E.C. 1.11.1.16), we found that a catalase-like activity surrounds the tips of growing rice plants. The results obtained by sampling the greenhouse rhizosphere (Table 1) suggest that in all cases catalase activity was associated with the rice roots. The differences between catalase levels in rice-planted soil and those in controls were highly significant ($F = 5935.5$ with 1 and 597 degrees of freedom). A similar experiment with sterilized soil and the controlled environment of a growth chamber gave similar results (Table 2).

In a subsequent experiment, rice seedlings were disinfested with NaClO for the removal of rice rhizosphere organisms, and the individual seedlings were grown for 10 days in cotton-stoppered tubes containing sterile Hoagland's solution (6). The seedlings were then removed and plated on nutrient agar in petri dishes. The plates were examined after 1 week for growth of contaminating organisms (contaminated tubes were eliminated from the test) and the tubes of growth solution were tested for catalase activity. The controls consisted of rice seedlings treated in the same manner as the test seedlings, but killed with propene oxide before placement in the nutrient solution. The catalase units per seedling of 16 uncontaminated rice seedlings after 10