

leave the insect rectum within a week. Whether the damselfly benefits from its symbiotic partner as a potential source of oxygen is unknown.

Symbiotic associations involving either damselflies or green euglenoids are virtually unknown. Lackey (4) mentions only the epizoid *Colacium* and the endozoic *Euglenamorphia hegneri* Wenrich. Michajlow (5) discusses euglenoids as possible parasites of the guts of copepods. Although not recorded as such, damselflies may, like the closely related dragonfly nymphs, act as an alternate host for trematode parasites (2) and might even carry the epizoid *Colacium cyclopicola* Gicklhorn which has been observed on copepods, cladocerans, ostracods, and larval water mites (6). We know of no other instance in which green species of euglenoid flagellates form a phycobiotic association with damselfly nymphs except where the latter's cuticle acts as a casual substrate for temporary attachment. We define symbiosis here as "a close, long-lasting association of dissimilar organisms" (7). The repeated occurrence of this relationship over three successive years and the seasonal synchrony constitute, we believe, valid evidence that the interaction of *Euglena* and the damselfly nymph represents a case of seasonal symbiosis.

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References and Notes

1. These ponds are located in Miller, Lake County, Indiana, about ¼ mile south of Lake Michigan.
2. P. S. Corbet, *A Biology of Dragonflies* (Quadrangle, Chicago, 1963), pp. 49, 56.
3. Dr. L. P. Johnson (Drake University) has kindly examined our living specimens and electron photomicrographs and agrees that their taxonomic position is uncertain. Our description of the species is in preparation.
4. J. Lackey, in *The Biology of Euglena*, D. E. Buetow, Ed. (Academic Press, New York, 1968), vol. 1, chap. 4.
5. W. Michajlow, *Acta Parasitol. Polon.* **13**, 313 (1965).
6. M. Gojdics, *The Genus Euglena* (Univ. of Wisconsin Press, Madison, 1953), p. 91.
7. V. Ahmadjian, *The Lichen Symbiosis* (Blaisdell, Waltham, Mass., 1967), p. 1.
8. Supported by a University of Illinois, Chicago Circle, Research Board grant to R.L.W. Dr. L. P. Johnson and Dr. R. B. Willey (University of Illinois, Chicago) conferred with us on various aspects of this work. D. Mucha provided essential technical assistance.

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Aspartylphenylalanine Methyl Ester:

A Low-Calorie Sweetener

Abstract. Sensory evaluation indicated that aspartylphenylalanine methyl ester is about 160 times sweeter than sucrose in aqueous solution. Blends of this dipeptide with (i) sodium saccharin, (ii) sodium saccharin and sucrose, and (iii) sodium saccharin, sucrose, and calcium cyclamate did not differ significantly from 4 or 12 percent sucrose in bitterness, off-flavors, or aftertaste.

Aspartylphenylalanine methyl ester (1) is about 160 times sweeter than sucrose in aqueous solution. This dipeptide does not differ significantly from sucrose in bitterness, aftertaste, off-flavors, or general acceptability in concentrations comparable in sweetness to 4 percent sucrose. Also, blends of the dipeptide with (i) sodium saccharin, (ii) sodium saccharin and sucrose, and (iii) sodium saccharin, sucrose, and calcium cyclamate do not differ significantly from 4 percent sucrose in bitterness, aftertaste, or off-flavors (Table 1).

Two and four percent sucrose solutions are approximately equal to 1 and 2 teaspoons of sugar per 8 ounces of liquid (2.1 and 4.2 g per 100 ml), respectively. Thus, this would be comparable to amounts of sugar commonly used in tea and coffee. Tea (2) had little effect on the sweetness of blends of the sweeteners at the concentrations used. However, slightly more dipeptide was required in tea than in water for sweetness equal to 2 percent sugar (Table 2).

Initially, the dipeptide in a concentration [(6.7 mg per 100 ml) × 10⁻²] slightly less sweet than 10 percent of sucrose was characterized by a marked off-flavor. However, at concentrations equal to 12 percent sucrose, blends were acceptable in flavor and did not differ significantly from sucrose in bitterness, off-flavors, or aftertaste. Twelve

percent sucrose, and the blend containing calcium cyclamate, were significantly preferred to the other two blends in flavor and general acceptability (Table 1). Because carbonated soft drinks range from 8 to 12 percent in sugar content (3), these findings are applicable to the beverage industry.

For the above evaluations, all solutions were prepared (weight to volume) at 20°C with odor-free distilled water. Blends of sweeteners were formulated so that approximately equal sweetness was contributed by each. Synergism was evident when solutions were formulated as though sweetness contributed by each compound was additive. Therefore, it was necessary to adjust sweetness by reducing the concentration of each compound in the blends.

Equivalents for sweetness were established by means of ranking tests with at least 20 judges. The following formula for chi square of ranks (4) was used for analysis of data from these tests:

$$\chi^2 = \frac{12}{np(p+1)} \times \frac{\text{sum}(\text{rank totals})^2 - 3n(p+1)}{p}$$

where p equals the number of treatments, n the number of replications, and d.f. equals $p-1$; 12 and 3 are constants. Sweetener solutions were reformulated and retested if they were significantly different from sucrose

Table 1. Mean ($n=20$) panel scores of flavor attributes of sweeteners and blends of sweeteners. Means followed by the same letter do not differ significantly within columns within concentrations of sweetness (6). Abbreviations are: S, sucrose; D, dipeptide; SS, sodium saccharin; and CC, calcium cyclamate.

Sweetener	Scale: 1 (low) to 9 (high)		Scale: 0 (none) to 3 (strong)		
	Flavor	General acceptability	Bitter	Off-flavor	Aftertaste
Sweetness equivalent to 4 percent sucrose					
S	6.4 ^a	6.0 ^a	0.5 ^a	0.1 ^a	0.4 ^a
D	5.3 ^b	5.3 ^a	0.8 ^a	0.2 ^a	0.8 ^a
D + SS	6.0 ^{ab}	5.7 ^a	0.6 ^a	0.3 ^a	0.7 ^a
D + SS + S	5.9 ^{ab}	5.8 ^a	0.4 ^a	0.1 ^a	0.7 ^a
D + SS + S + CC	5.6 ^{ab}	5.4 ^a	0.7 ^a	0.1 ^a	0.8 ^a
Sweetness equivalent to 12 percent sucrose					
S	7.1 ^a	7.1 ^a	0.6 ^{ab}	0.1 ^a	1.0 ^{ab}
D + SS	6.3 ^b	6.0 ^b	1.0 ^a	0.2 ^a	1.5 ^a
D + SS + S	5.8 ^b	6.0 ^b	0.8 ^{ab}	0.1 ^a	1.1 ^{ab}
D + SS + S + CC	7.3 ^a	7.0 ^a	0.4 ^b	0.2 ^a	0.8 ^b

Table 2. Percent concentrations of sweeteners and blends of sweeteners equivalent ($n > 20$, $P < .05$) to selected sucrose concentrations in water and in tea. Abbreviations as in Table 1.

Sweet- ness equiv- alent (grams of sucrose per 100 ml)	Sweetener [(g/100 ml) $\times 10^{-2}$]				
	D	+	SS	+	S + CC
<i>In water</i>					
2	1.10				
4	2.50				
4	1.00		0.20		
8	2.00		0.60		
12	3.00		1.50		
4	0.85		0.15		130
12	1.60		0.80		400
4	0.55		0.10		100
12	1.00		0.50		200
					3.30
					7.50
<i>In tea</i>					
2	1.25				
4	2.50				
4	1.00		0.20		
8	2.00		0.60		

($P < .05$). By these tests concentrations of sweeteners [(grams per 100 ml) $\times 10^{-2}$] equivalent to 2, 4, 8, and 12 percent sucrose in water and 2, 4, and 8 percent sucrose in tea were determined (Table 2).

For descriptive analysis of the dipeptide solutions and sweetener blends which were equal in sweetness to 4 and 12 percent sucrose, there was a panel of ten members. Analyses of variance (5) were applied to these data; Duncan's (6) new multiple range test was used to locate differences among means (Table 1).

These studies indicate that aspartyl-phenylalanine methyl ester has potential as a low-calorie sweetener. Because

slightly more dipeptide was required for sweetening tea than water, it is likely that changes will be necessary in applying these findings to different beverages or foods. It is not possible to predict whether the dipeptide or the blends of sweeteners will be more or less effective in sweetening, or more or less acceptable, in foods or beverages other than water and tea. Even in water and tea, the flavor attributes might change significantly if ratios of the sweeteners were varied and if sweetness contributed by the individual components was unequal. As with most sweeteners, the dipeptide was more acceptable and relatively sweeter in low concentrations than in high. Hence, use of blends of sweeteners reduces the amount of each needed for desired sweetness, especially when there is synergism among the compounds. Also, by maintaining low concentrations of sweetening agents, problems of toxicity are less likely.

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References and Notes

1. Fermco Lab., Div. of G. D. Searle and Co., Chicago, Ill. 60680.
2. Nestea (0.134 percent), The Nestle Co., Inc., White Plains, N.Y. 10605.
3. B. K. Watt and A. L. Merrill, *Composition of Foods* (USDA Handbook No. 8) (Department of Agriculture, Washington, D.C., 1963), p. 16.
4. M. Friedman, *J. Amer. Stat. Ass.* **32**, 675 (1937).
5. G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, ed. 6, 1967), pp. 260-267.
6. D. B. Duncan, *Biometrics* **11**, 1 (1955).
7. Contribution from the Missouri Agricultural Experiment Station Journal series No. 6006.

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Diurnal Variation of Spontaneous Uterine Activity in Nonpregnant Primates (*Macaca mulatta*)

Abstract. *Diurnal variations in the average intrauterine pressure and in the average frequency of contraction were demonstrated in four nonpregnant rhesus monkeys. Uterine activity is lowest between 0400 and 0700 hours and highest between 1400 and 1700 hours. There is statistically significant correlation between uterine activity and diurnal variation in core temperature.*

Variations in the contractile pattern that occur without apparent external cause are a characteristic of the spontaneous activity of uterine muscle. This phenomenon has been observed during observations of excised myometrial strips in vitro (1) and during studies of nonpregnant human (2) and subhuman primate uteri in vivo (3).

We used four nonpregnant rhesus monkeys (*Macaca mulatta*), with documented regular menstrual cycles, to delineate some of the factors that produce these alterations of spontaneous uterine motility. After the animals were anesthetized, an open-minded, fluid-filled catheter was introduced, either transcervically or transmyometrially,

into the uterine cavity and secured to the uterus with a silk suture. As soon as the operation was complete, the animal was placed in a restraining chair and allowed to recover from anesthesia. Three to five days after surgery, the catheter was flushed with heparinized saline and connected by way of a pressure transducer to a recording polygraph. Spontaneous alterations in pressure within the uterine cavity were monitored continuously until completion of the observations.

On-line electronic integration of the pressure signal was used to facilitate quantitative evaluation of the changes in motility patterns. This measure of the area under the pressure curve encompasses frequency, amplitude, and duration of contraction, as well as the base line pressure during uterine diastole and permits measurement of total uterine activity in physical units for statistical comparison. Average intrauterine pressure was calculated by dividing the total uterine activity by a selected time interval. Frequency of contraction was expressed as the number of complete contraction and relaxation complexes occurring per minute. A contraction was defined as having occurred when the pressure rose 10 mm-Hg above the preceding base line diastolic pressure and subsequently fell below this arbitrarily selected value.

Three animals were maintained in a controlled environment with constant temperature and alternating 12-hour cycles of light and dark, changing at 0500 and 1700 hours. The fourth animal, M-14, was maintained under natural conditions of light and dark (sunrise approximately 0700 hours; sunset approximately 1700 hours).

Flushing of the catheter system and checks of the accuracy of the recording system were the only special procedures necessary. Periodically, vaginal smears were obtained for cytological evaluation. In certain instances deep body temperature was continuously recorded by a thermistor placed in the abdominal cavity.

Uterine activity was measured in the four monkeys for a total of 173 days. Figure 1 shows tracings typical of uterine contractile patterns obtained during menstrual, proliferative, and secretory phases of the same menstrual cycle. Tracings obtained between 0600 and 0800, 1200 and 1400, and 2200 and 2400 hours are shown for each cycle phase. The overlay is a graphic presentation of the calculated data. On day 1 of the cycle, mean intrauterine