

15. It is presumed that volatile components of the extracts of the 1972 laboratory-reared females were lost because of evaporation during storage. These are the same samples that we analyzed previously and reported in (1).
16. The ozonide was decomposed with a small excess of triphenylphosphine in methylene chloride solution, in order to avoid chromatographic interference from trace contaminants in this reagent. The reaction mixture was maintained at 0°C until analysis, then methylene chloride was carefully evaporated (not to dryness) under N₂, reconstituted in hexane, and concentrated to approximately 10 µl.
17. M. Beroza and B. A. Bierl, *Anal. Chem.* **39**, 1131 (1967); *Mikrochim. Acta* **4**, 720 (1969).
18. Ozonolysis blanks were clean except for trace impurities eluting from OV-1 well before the region of interest.
19. Microozonolyses at levels below 50 ng per component yield product mixtures in which background and artifact chromatographic peaks become significant. Analysis of a natural extract for minor components would be uninformative at this level.
20. The carrier gas for GC analysis of ozonolysis products was He (30 ml/min) for OV-1 programmed from 100° to 170°C at a rate of 6°C per minute, or at a constant temperature of 140°C. GC-MS analyses of ozonolysis products were conducted with methane (30 ml/min) as the carrier-reagent, as above. The temperature programming technique allowed practical detection of aldehydes of chain length eight or greater and acetoxyaldehydes of chain length six or greater. Thus, one ozonolysis fragment would be detected from any monounsaturated 14-carbon acetate isomer.
21. Reconstructed mass chromatograms for any *m/e* can be recalled by the data system from stored total mass spectra. These can be plotted in the same format as mass fragmentograms. However, mass fragmentography analyzes and stores only a few ions, disregarding the remainder of the mass range. For a more complete description of mass fragmentography, see (1) and (6).
22. The retention times (spectrum numbers) for the *m/e* 241 and 269 leaf extract components, as well as standard tetradecenyl acetates, are different for CI and EI mass spectra in Fig. 3 because of differences in chromatographic conditions on these instruments.
23. The only GC-MS ionization mode available in this laboratory during the original identification of pheromones in plants (1) was EI.
24. In CI spectra the *m/e* 241 and 269 leaf components give certain fragment ions similar to diagnostic tetradecenyl acetate ions. For example, the 269 component has a minor *m/e* 61, a strong *m/e* 195 fragment, and a strong signal at *m/e* 253 which produces a trace isotope pattern extending to *m/e* 255.
25. Saturated tetradecyl acetate does appear to be a true leaf extract component when analyzed before the DEGS collection which is timed to remove most of it.
26. The carrier gas used for collection from QF-1 was He (30 ml/min at 160°C, isothermal).
27. The mass spectrum of this leaf extract component shows a prominent (M + 1 - 32)⁺ characteristic of fatty acid methyl esters, and would be consistent with a methyl pentadecenoate (C₁₆H₃₂COOCH₃). Other plant extract components may be due to a homologous series of fatty esters.
28. No leaf tetradecenyl acetates were detected by GC-MS analysis of 50 g of plant material. One nanogram was an approximate lower sensitivity limit for adequate full CI mass spectra including (M + 1)⁺, (M + 29)⁺, and (M + 41)⁺. However, trace amounts below 100 pg of tetradecenyl acetates would be indicated by reconstructed mass chromatograms of prominent fragments such as *m/e* 195 and 61.
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30. For helpful discussions we thank R. D. Minard, M. Shamma, and other members of the Department of Chemistry and R. O. Mumma of the Department of Entomology and Pesticide Research Laboratory, Pennsylvania State University. We also thank K. W. Dillan for assistance in the purification of leaf extracts.

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The Stomach as a Site for Rapid Nutrient Reinforcement Sensors

Abstract. *Rats with inflated cuffs placed around the pyloric sphincter were given a choice between two nonnutritive solutions. Ingestion of one solution was paired with nutritive intragastric injections, and ingestion of the other was paired with saline injections. The preference of rats for the nutrient-paired flavors indicates that the stomach alone can rapidly detect the arrival of nutritive substances.*

When predigested milk is injected into the stomachs of rats that are choosing between two samples of flavored water, the rats will choose the flavor paired with the nutrient (1). It is unlikely that this result is due to stomach distention, because the effect occurs when an intragastric saline load of equal volume is injected into the stomach when the other sample is chosen. Nor is the rewarding effect due to absorption. Each daily choice session lasts only 10 minutes, and most rats sample both flavors in each session (Table 1). Since the effect does not occur when glucose is substituted for the milk, the rats probably do not regurgitate and thus taste the flavor. Although the predigested milk is injected directly into the stomach, the site of action could actually be in the duodenum, as a considerable volume immediately enters the duodenum at this time (2). We have attempted to determine whether nutrient can be detected by the stomach or whether nutrient is detected by the duodenum alone; we now show, for what we believe to be the first time, that such detection does occur in the stomach. In order to distinguish between these alternatives, an inflatable cuff was implanted and placed around the pyloric sphincter of rats. The cuff was inflated so as to close off completely the entrance to the duodenum while rats were allowed for 10 minutes to choose between two flavored non-nutritive solutions. When the rat chose and drank one solution, an equal volume of predigested milk was injected into the stomach. When the rat drank the other flavored solution, an equal volume of saline was also injected into the stomach.

Table 1. Mean intake [together with standard error of the mean (S.E.M.)] of flavor paired with predigested milk injection and of flavor paired with saline injection, and the number of rats (out of eight) sampling both flavors.

Day	Intake				Rats (No.)
	Nutrient- paired flavor (ml)		Saline- paired flavor (ml)		
	Mean	S.E.M.	Mean	S.E.M.	
1	4.29	0.95	2.31	0.98	7
2	4.15	1.01	2.22	0.81	8
3	5.40	0.65	1.62	0.57	8
4	5.97	0.52	0.95	0.37	8
5	6.32	0.35	0.67	0.27	6

The subjects were 15 naive male rats (Sprague-Dawley) weighing between 350 and 450 g at the time of surgery. Ten rats served as experimental animals and five as donors. In the experimental rats, two stomach tubes were implanted [according to the method of Deutsch and Hardy (3)] and an inflatable cuff. In the donors, one stomach tube was implanted. Animals were allowed to recover for 1 week after surgery. During this period, milk (Carnation evaporated) and water were freely available.

After recovery, donor rats were put on a 22-hour food and water deprivation schedule. They were allowed access to milk for 2 hours every day.

At the same time, experimental rats were also put on a food and water deprivation schedule. Every morning two burettes were filled with water and their drinking spouts were inserted into each cage for 10 minutes. One hour later, subjects were given 8 g solid food. After daily watering and feeding, each rat's intragastric fistulae were cleared with 1 ml water. At this time the cuff was temporarily inflated in order to accustom animals to the experimental procedure. No apparent physical discomfort was exhibited by the animals. After rats had shown a stable water intake for at least 3 days and had sampled from both spouts, preliminary training ended.

Once the experimental conditions began, the daily routine was as follows: First, donors were allowed to drink milk. When they had stopped drinking for 5 minutes, milk was pumped out through the intragastric fistulae and cooled to room temperature. Second, the cuffs of the experimental animals were inflated. These rats were then given a choice between two flavored solutions: banana (0.5 percent banana flavoring, Schilling) and almond (0.5 percent almond flavoring, Schilling). When half the rats drank the banana flavor, predigested milk was injected into their stomachs at the rate at which they drank. When these rats drank the almond flavor, physiological saline (Tis-u-sol) was injected. For the remaining rats, the pairings were reversed. There were five 10-minute sessions, one each day for 5 days.

After this phase, each of the ten experimental rats was anesthetized as before, a laparotomy was performed, and the infla-

tion of the cuff was verified. Two rats were found to have nonfunctioning cuffs; their scores were therefore excluded from the analysis. The analysis of variance and specific comparisons were based on the other eight rats. The rats clearly preferred the nutrient paired flavor [$F(1,7) = 17.35, P < .01$] (Table 1).

It seems that the stomach can recognize some components of food and signal their arrival rapidly to the central nervous system, where such messages produce reinforcement. So far, the presence of nutrient sensors in the duodenum, signaling to the brain via the release of cholecystokinin, has been postulated (4). The presence of nutrient sensors

above the level of the duodenum must also be considered. The mechanism by which the sensors in the stomach transmit their signals to the central nervous system remains to be elucidated.

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Sea Straits and Glacial Periods in the Red Sea

Deuser *et al.* (1) compared oxygen isotopes in tests of foraminifera from sediments of the Gulf of Aden with those from the Red Sea. In glaciated periods they found an ^{18}O enrichment of 2 per mil in the Red Sea tests, which corresponds to an excess salinity of 6 to 7 per mil in the Red Sea over the Gulf of Aden. Today this excess salinity is only 3 per mil. Deuser *et al.* concluded that "during the periods of maximum glaciation the climate in the area of the Red Sea was, on the average, considerably drier than today."

This conclusion seems to be in conflict with what is known about flow through sea straits. Deuser *et al.* stated that lowering sea level would reduce the exchange between the Gulf of Aden and the Red Sea. However, the implication of such a reduction would invalidate their conclusion about the paleoclimate of the Red Sea.

The Red Sea is in a state of frictional overmixing such that the exchange through the Strait of Bab al Mandab is limited by critical Froude conditions (2). The salinity difference $\Delta s = s_2 - s_1$ between the discharge from the Red Sea, s_2 , and the inflow to the Red Sea from the Gulf of Aden, s_1 , is related to the bathymetry of the strait and the excess evaporation over the Red Sea according to

$$\Delta s \propto q^{2/3} L^{1/3} Y^{-2/3} D^{-4/3}$$

or

$$\frac{q^*}{q} = \left(\frac{\Delta s^*}{\Delta s} \right)^{3/2} \left(\frac{D^*}{D} \right)^2 \left(\frac{Y^*}{Y} \right) \left(\frac{L}{L^*} \right)^{1/2}$$

Here q is the excess evaporation over the Red Sea, and L , D , and Y are the length, depth, and width of the strait. Quantities with asterisks refer to glacial periods, and those without asterisks re-

fer to the present. The salinity (or ^{18}O) differences are thus more sensitive to changes in depth than to changes in the evaporation rate.

For present-day conditions one may characterize the Strait of Bab al Mandab by the parameters (2) $L = 160$ km, $Y = 18$ km, $D = 180$ m, $q = 28000$ m³/sec, and $\Delta s = 0.003$. The sea level lowering at the maximum of the glacial period was 130 m (3), and on the average I will conservatively assume a lowering of 80 m. The glacial parameters in the above equation can then be approximated by $L^* = 160$ km, $Y^* = 12$ km, and $D^* = 100$ m. Adopting the estimate $\Delta s^* = 0.007$ of Deuser *et al.*, I obtain

$$q^*/q = 0.73$$

Thus, it seems more likely that during glacial periods the climate of the Red Sea area was similar to or somewhat more humid than that of today.

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I am grateful to Assaf for applying his model for the water exchange through straits (1) to our data in order to check the implications for the Red Sea climate during glacial times (2). I am not convinced that the model with its implicit and explicit assumptions and simplifications can do justice to the complexities of nature, but I will not argue about its applicability in this space nor take issue with Assaf's choice of dimensions for the Strait of Bab al Mandab (3). However, even if the value of 0.73 for the ratio of glacial to present excess evaporation over the Red Sea were correct, Assaf's conclusion is not valid. An 80-m drop of sea level during glacial times reduced the surface area of the Red Sea by 37 percent (4). Therefore, the evaporation had to take place over an area a^* which was only 63 percent of the present surface area a . The climatically significant quantity in the context of our report (2) is excess evaporation per unit area of sea surface, e , and not the net influx, q , of water through the Strait of Bab al Mandab. Using Assaf's asterisk notation and his influx ratio of 0.73, I obtain

$$\frac{e^*}{e} = \frac{q^* a}{q a^*} = \frac{0.73}{0.63} = 1.16$$

Thus, whatever the merit of the model calculations, it still seems likely that during glacial periods the climate in the Red Sea was drier than today.

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3. For the most detailed bathymetric survey of the sill area at the southern end of the Red Sea, see F. Werner and K. Lange, *Geol. Jahrb. Reihe D* **13**, 125 (1975).
4. I calculated this value by planimetry of the areas within the coastline and the 50- and 100-m depth contours on the map of M. Pfannenstiel and G. Giermann [in S. A. Morcos, *Oceanogr. Mar. Biol. Annu. Rev.* **8**, 73 (1970)] and interpolating the 80-m value.
5. Inasmuch as I was responsible for drawing the conclusion in our jointly authored report (2) which was questioned by Assaf, I assume responsibility for this reply. Supported by NSF grant OCE73-06586.

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Galilean Satellites: Anomalous Temperatures Disputed

In a recent report, Gross (1) argues that the observed infrared brightness temperatures of the Galilean satellites are significantly higher than temperatures calculated on the assumption that these largest satellites of Jupiter are in

equilibrium with the incident sunlight. He therefore suggests that their surfaces are being heated above equilibrium values by energetic atomic particles in the Jovian magnetosphere but notes that the observed particle fluxes are about an or-