

# Acute Blockade of Gastric Emptying and Meal Size in Rats

E. A. RAUHOFFER, G. P. SMITH<sup>1</sup> AND J. GIBBS

Department of Psychiatry, Cornell University Medical College, and Edward W. Bourne Behavioral Research Laboratory, New York Hospital–Cornell Medical Center, White Plains, NY 10605

Received 7 December 1992

RAUHOFFER, E. A., G. P. SMITH AND J. GIBBS. *Acute blockade of gastric emptying and meal size in rats.* PHYSIOL BEHAV 54(5) 881–884, 1993.—To investigate the functional capacity of the combination of pregastric and gastric satiating stimuli for the control of meal size, rats were surgically implanted with a pyloric cuff that could be inflated to prevent gastric emptying. After 0.5 or 19 h of food deprivation, rats were given access for 30 min to three concentrations of sucrose (0.8, 0.4, and 0.2 M) and of corn oil emulsions (100%, 50%, and 25%) in descending order. There were only two significant changes in intake between cuff-open and cuff-closed tests and both occurred after 19 h of food deprivation: 1) meal size of 50% corn oil with the cuff closed was significantly larger than meal size of 50% corn oil with the cuff open on the preceding day; 2) meal size of 0.8 M sucrose with the cuff closed was significantly larger than meal size with the cuff open on the next test day. These results provide new experimental evidence that when ingested food is prevented from emptying from the stomach acutely, the satiating effect of food stimuli acting at pregastric and gastric sites is sufficient to produce meals of normal size under many, but not all, experimental conditions.

Postprandial satiety	Food intake	Pyloric cuff	Pregastric satiety	Sucrose	Gastric satiety	Corn oil
----------------------	-------------	--------------	--------------------	---------	-----------------	----------

WHEN ingested food is prevented from emptying from the stomach by a noose or inflatable cuff around the pylorus, the size of a liquid meal is not significantly different from the meal size observed when gastric emptying occurs normally (3–5,7,8). The ability of the combination of pregastric and gastric stimuli to control meal size has been observed after 3 or 15 h of food deprivation and with high-carbohydrate, low-fat diets, milk, and a 50% corn oil emulsion.

To investigate the functional capacity of these pregastric and gastric controls further, we tested rats with the pyloric cuff inflated or deflated and varied the diet and the interval of food deprivation. The diets were three concentrations of sucrose and three concentrations of corn oil emulsions. Intakes of these diets were measured after 0.5 or 19 h of food deprivation. A preliminary report has appeared (10).

## METHOD

Male Sprague–Dawley rats (300–500 g) were housed individually in wire bottom, hanging cages and maintained on a 12:12 light:dark cycle (lights off at 1900) at a temperature of  $22 \pm 1^\circ\text{C}$ . Except for deprivation periods, rats had ad lib access to pelleted food (Purina Rat Chow 5001). Tap water was freely available except during testing.

## Surgery

Rats were surgically implanted with a pyloric cuff that could be inflated to prevent gastric emptying (11). Each pyloric cuff was constructed using a  $20 \times 40$  mm piece of Silastic sheeting (Dow Corning #500-1) 0.005" thick, a  $6 \times 40$  mm piece of Silastic sheeting (Dow Corning #500-3) 0.010" thick, and a 20-cm piece of 0.030" i.d.  $\times$  0.065" o.d. Silastic tubing (Dow Corning #602-175). The edges of the  $20 \times 40$  mm piece of Silastic sheeting were covered with silicon medical adhesive (Dow Corning #891). The Silastic tubing was placed in the center of the  $20 \times 40$  mm piece. The  $6 \times 40$  mm piece was placed directly over the tube and lightly pressed into the glued edges of the underlying larger sheet. Each edge of the larger piece was folded on top of the smaller piece, creating an air-tight pocket. Adhesive was placed over the seam where the larger sheeting overlapped and at both ends of the cuff.

Surgery was performed under surgical anesthesia produced by a mixture of chloral hydrate and pentobarbital (3 ml/kg, IP). An incision was made at the xyphoid process and extended 5 cm caudally. The stomach was located and the pylorus was identified. A curved hemostat was used to separate the pylorus from the underlying tissues and blood vessels. Extreme care was used to avoid damaging blood vessels or nerves. Once the hemostat was directly under the pylorus, it was used to pull the pyloric cuff under the pylorus. The ends of the pyloric cuff were

<sup>1</sup> Requests for reprints should be addressed to G. P. Smith, Edward W. Bourne Behavioral Research Laboratory, New York Hospital–Cornell Medical Center, White Plains, NY 10605.



sutured (Ethicon 3-0 taper needle with silk suture #K843H) together lateral to the pyloric cuff tube. Care was used to avoid puncturing the tube with the needle when these sutures were made. Saline (0.15 M NaCl) was infused through the end of the pyloric cuff tube to determine the volume needed to close the pylorus completely. The volume was  $\leq 0.7$  ml. The specific volume required to close the pylorus at surgery in a rat was used in all tests with that rat.

After the stomach and other viscera were returned to the abdominal cavity, the pyloric cuff tube was brought through the center of a piece of Marlex mesh (Bard #011266) cut to a diameter of 1.5 cm. A hole was made through the abdominal musculature approximately 4 cm to the left of the midline incision for the tubing to be brought through. The Marlex mesh was sutured (3-0 silk) to the peritoneal surface of the abdominal muscles. Then a midline incision ( $\sim 1$  cm) was made on the dorsal surface of the neck. A 19-ga trocar was pushed SC from the neck toward the pyloric cuff tube. The end of the tube was fitted onto the trocar and pulled through the neck incision.

The pyloric cuff tube was pushed through a larger diameter Silastic tubing (0.078" i.d.  $\times$  0.125" o.d., Dow Corning #602-305). This outer tubing protected the pyloric cuff tube from damage by the rat. The larger tubing was attached through the center of a 1-cm piece of Marlex mesh by medical adhesive (Dow Corning, #891). The Marlex mesh was sutured (3-0 silk) SC inside the dorsal neck incision to provide a stable attachment for the outer tube. Then the incision was closed with wound clips or interrupted, 3-0 silk sutures.

#### Test Procedures

Two weeks were allowed for recovery from surgery. Rats were tested first after 19 h of food deprivation and then after 0.5 h of food deprivation. In both of these deprivation conditions, intakes were measured after presentation of a sucrose solution (0.2, 0.4, or 0.8 M) or corn oil-water emulsion (25%, 50%, or 100%). Corn oil emulsions were made by mixing 100% corn oil (Mazola Co.) with deionized water and Tween 80 (Sigma), 0.75 ml/100 ml emulsion. Emulsions were mixed just before presentation and no significant separation occurred during the 30-min test. We have previously demonstrated that rats do not discriminate this concentration of Tween 80 (9).

Tests began after intakes of a specific sucrose solution or corn oil emulsion stabilized with the pyloric cuff open. All tests were run over 3 consecutive days: day 1, cuff open (cuff was deflated and gastric emptying could occur); day 2, cuff closed (cuff was inflated and gastric emptying was prevented); and day 3, cuff open.

On each test day, in the 0.5-h deprived condition, food was removed at 1030. In the 19-h deprived condition, food was removed at 1600 on the preceding day. Water was removed at 1045 in all tests. Then each rat was removed from its cage, a 1-ml bacteriostatic, saline-filled syringe with an 18-ga luer stub adapter was attached to the tube of the pyloric cuff and the predetermined volume that inflated the cuff sufficiently to close the pylorus was infused. The tube was occluded by clamping a curved hemostat approximately 5 mm from its end. Then an 18-ga bent needle, with its edges cut and filed, and filled with Critoseal (Lancet, St. Louis), was used to plug the outer end of the tube.

On a cuff-open test day, the rats were handled similarly except no saline was infused into the pyloric cuff (it remained deflated). All tests occurred between 1100 and 1130. Intakes were recorded at 3-min intervals during the 30-min test. The 30-min intake

was equal to meal size because all rats terminated eating within this interval. At 1140 the rats were removed from their cages. Using the curved, Silastic-covered hemostat, the tube was pinched below the Critoseal-filled needle. Once the bent needle and hemostat were removed, a 1-ml syringe was used to withdraw the saline from the inflated cuff. The volume withdrawn had to equal the volume infused for the test to be considered valid. A cuff-open rat was handled in the same manner. At 1200 pelleted food and water were returned.

In both deprivation conditions, rats were tested with descending concentrations of sucrose (0.8, 0.4, and 0.2 M) and then descending concentrations of corn oil emulsions (100%, 50%, and 25%). Due to technical problems with the cuff or the failure of rats to achieve stable intakes of some stimuli after 0.5 h of food deprivation, the number of rats from which data were obtained varied and are listed in Table 1.

At the end of testing, rats were anesthetized with a mixture of chloral hydrate and pentobarbital (3 ml/kg, IP) and the pyloric cuff was exposed through a midline abdominal incision. Then the cuff was inflated with the specific volume of saline used throughout the tests. If the inflated cuff closed off the pylorus, the data from that rat were considered valid. If the inflated cuff did not close the pylorus completely or if the cuff leaked, the data from that rat were discarded.

#### Data Analysis

Intakes (ml/30 min) of each concentration of sucrose and corn oil after a specific deprivation were analyzed across the 3 test days by a one-way ANOVA using a repeated measures design. Post hoc tests of significant differences between test days were done using Tukey's HSD test. All analyses were performed using SAS Statistical programs (Cary, NC).

#### RESULTS

There were only two significant changes in the 12 test conditions. Acute blockade of gastric emptying by inflating the pyloric cuff increased intake of 50% corn oil significantly after 19 h of food deprivation compared to the intake on the preceding day, when the cuff was not inflated and gastric emptying occurred normally (Fig. 1). The only other statistically significant difference was that intake of 0.8 M sucrose with the cuff closed after 19 h of food deprivation was significantly larger than the intake on the next day, when the cuff was open (Fig. 2). Inspection of intakes at 3-min intervals did not reveal any significant differ-

TABLE 1  
NUMBER OF RATS TESTED  
IN EACH CONDITION

Test Nutrient	Hours of Food Deprivation	
	0.5	19.0
Sucrose		
0.8 M	8	11
0.4 M	5	10
0.2 M	15	9
Corn oil		
100%	3	8
50%	8	8
25%	11	8



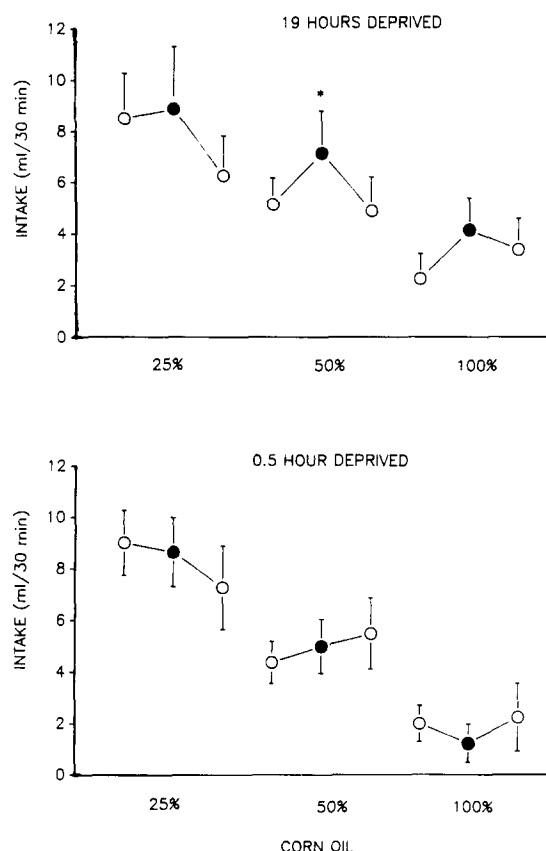


FIG. 1. Mean  $\pm$  SE ml/30 min of 25%, 50%, or 100% corn oil ingested with a pyloric cuff open (open circles) and a pyloric cuff closed (filled circles) on sequential test days after 19 or 0.5 h of food deprivation. Intake with a pyloric cuff closed was significantly larger than intake with a pyloric cuff open, \* $p < 0.05$ .

ences between cuff-open and cuff-closed conditions in any of the other tests (data not presented).

The intakes of sucrose and corn oil were an inverse function of concentration in both deprivation conditions when the pyloric cuff was not inflated (Figs. 1 and 2). This adjustment of intake also occurred when the cuff was inflated and gastric emptying did not occur.

#### DISCUSSION

In general, these results confirm previous reports (3-5,7,8) that meal size does not change significantly when gastric emptying of ingested food is prevented by acute mechanical closure of the pylorus. The results also extend the previous reports in two ways. First, they demonstrate an exception to the general rule in that rats ate significantly more 50% corn oil when the cuff was closed than when it was open after 19 h of food deprivation (Fig. 1). Although this result was clear under our conditions, its significance as a failure of pregastric and gastric satiating mechanisms to control meal size of 50% corn oil within normal limits depends upon how often it is observed in other experimental conditions.

The second way in which the results extend previous reports is the demonstration of the capacity of pregastric and gastric

food stimuli to control meal size of three different concentrations of sucrose solutions and of corn oil emulsions under two deprivation conditions, the most thorough test of these controls that has been carried out. It should be noted, however, that duration of food deprivation did not produce a significant effect on the intake of sucrose solutions or corn oil emulsions under our conditions. This may limit the generalizability of our results.

Note that our experimental design was such that the adjustment of intake to a change in concentration was made with the cuff open before a cuff-closed test occurred. Thus, every concentration of sucrose and corn oil tested in the cuff-closed condition had become familiar to the rat prior to a cuff-closed test. These results are consistent with the hypothesis of Deutsch that gastric stimuli for the control of meal size are most potent when the diet is familiar (2).

The relative quantitative contribution of pregastric and gastric satiating mechanisms to the control of intake in the cuff-closed conditions cannot be determined from our results. Although Deutsch concludes that when the diet is familiar, gastric satiating mechanisms exert the major, perhaps the sole, control, Davis et al. (1) recently demonstrated that the satiating effect of gastric stimuli accounted for some, but not all, of the control of meal size of 0.8 M sucrose when the cuff was closed. The relevant observation was that when ingested sucrose drained out through an open gastric cannula in a cuff-closed test, the intake doubled,

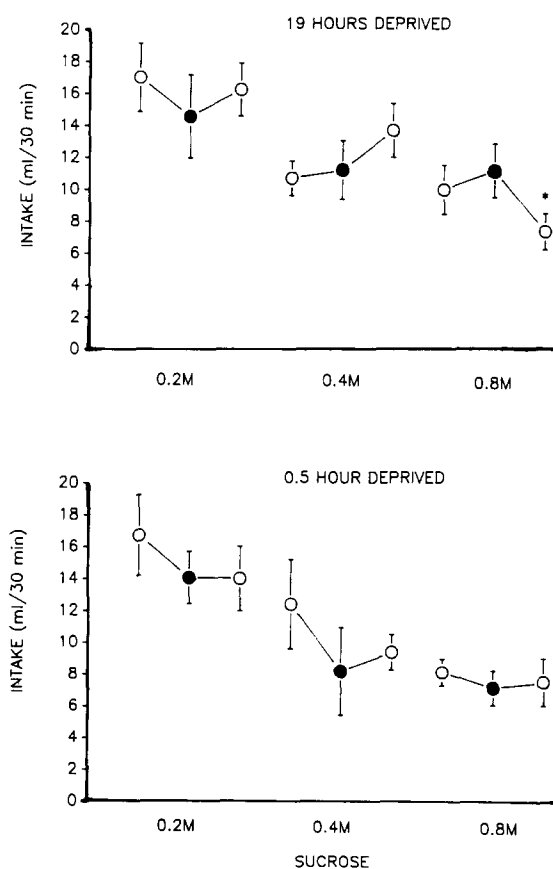


FIG. 2. Mean  $\pm$  SE ml/30 min of 0.2, 0.4, or 0.8 M sucrose ingested with a pyloric cuff open (open circles) and with a pyloric cuff closed (filled circles) on sequential test days after 19 or 0.5 h of food deprivation. Intake with a cuff open was significantly smaller than intake with a cuff closed, \* $p < 0.05$ .



and then eating terminated. The termination of eating in this situation in which the 0.8 M sucrose was familiar was probably due to pregastric satiating stimuli, although some chemical stimulation of the gastric mucosa occurred. The efficacy of pregastric stimuli to terminate eating after meal size had approximately doubled had been previously reported in rats after 3 h of food deprivation (6).

Thus, these results provide new evidence concerning the functional capacity of pregastric and gastric stimuli to control

meal size within normal limits. The adequate stimuli, peripheral and central mechanisms, and the relative potencies of pregastric and gastric satiating stimuli remain to be determined.

#### ACKNOWLEDGEMENTS

We thank Dr. Danielle Greenberg for statistical advice and Mrs. Jane Magnetti for processing the manuscript. This work was supported by MH15455 and by MH00149 (G.P.S.).

#### REFERENCES

1. Davis, J. D.; Smith, G. P.; Miesner, J. Postpyloric stimuli are necessary for the normal control of meal size in real and sham feeding rats. *Am. J. Physiol.* (in press).
2. Deutsch, J. A. Food intake: Gastric factors. In: Stricker, E. M., ed. *Handbook of behavioral neurobiology*. New York: Plenum Press; 1990:151-182.
3. Deutsch, J. A.; Gonzalez, M. F. Gastric nutrient content signals satiety. *Behav. Biol.* 30:113-116; 1980.
4. Deutsch, J. A.; Young, W. G.; Kalogeris, T. J. The stomach signals satiety. *Science* 201:165-167; 1978.
5. Gonzalez, M. F.; Deutsch, J. A. Vagotomy abolishes cues of satiety produced by gastric distention. *Science* 212:1283-1284; 1981.
6. Kraly, F. S.; Carty, W. J.; Smith, G. P. Effect of pregastric food stimuli on meal size and intermeal interval in the rat. *Physiol. Behav.* 20:779-784; 1978.
7. Kraly, F. S.; Gibbs, J. Vagotomy fails to block the satiating effect of food in the stomach. *Physiol. Behav.* 24:1007-1010; 1980.
8. Kraly, F. S.; Smith, G. P. Combined pregastric and gastric stimulation by food is sufficient for normal meal size. *Physiol. Behav.* 21:405-408; 1978.
9. Mindell, S.; Smith, G. P.; Greenberg, D. Corn oil and mineral oil stimulate sham feeding in rats. *Physiol. Behav.* 48:283-287; 1990.
10. Rauhofer, E. A.; Smith, G. P.; Gibbs, J. Prevention of gastric emptying does not alter food intake. *Proc. East. Psychol. Assoc.* 62:10; 1991.
11. Young, W. G.; Deutsch, J. A. The construction and surgical implantation of gastric catheters and a pyloric cuff. *J. Neurosci. Methods* 3:377-384; 1981.