Gastric negative feedback produced by volume and nutrient during a meal in rats

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Gastric negative feedback produced by volume and nutrient during a meal in rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1201–R1214, 2001.—To investigate the gastric negative-feedback control of eating during a meal, we implanted male rats with pyloric cuffs and gastric catheters and gave them access to sweet milk for 30 min after overnight deprivation. Ingested milk and infused milk or saline were confined to the stomach because the pyloric cuffs were closed in all tests. Rats received five consecutive tests with no gastric infusion or with infusions of 3, 6, or 12 ml of milk or saline during the first 6 min of the test meal. Only 12-ml infusions decreased intake significantly compared with no infusion. Because 12 ml of saline inhibited intake as much as 12 ml of milk, the decreased intake was due to volume or rate of infusion, not nutrient. Although infusions of 3 and 6 ml of milk did not decrease intake, they decreased the number of licks after the infusions significantly more than equal volumes of saline. Thus a large volume or rapid rate of gastric infusion decreases intake, and some other aspect of small milk infusions decreases the rate of licking.

satiation; control of meal size; food intake; pyloric cuff; microstructure of licking

IT IS WIDELY ACCEPTED THAT ingested food in the stomach provides a negative-feedback signal for the termination of eating and the control of meal size (9, 27). This conclusion was originally based on the inhibition of intake produced by the presence of a balloon or delivery of a preload into the stomach (28). This evidence was problematic, however, because a balloon does not have the physical and chemical attributes of ingested food, and a gastric preload empties rapidly from the stomach (18) so that at least some of its satiating effect is probably due to stimulation of small intestinal mechanisms (28).

These limitations have been overcome by confining ingested food to the stomach by closing the pylorus with a noose (15) or, more frequently, with an inflatable pyloric cuff (29) just before intake tests. Pyloric closure prevents gastric emptying and duodenal stimulation while allowing the physical and chemical attributes of the ingested food to act in the stomach. Because pyloric closure is reversible, intake with the pylorus closed can be compared with intake with the pylorus open in the same rats on different days.

When this comparison was made under a number of experimental conditions, intake was equal when the pylorus was closed or open (14, 20, 21, 24, 26). When intake with the pylorus open was relatively large, however, intake when the pylorus was closed was significantly smaller than when it was open (4, 7, 8, 12).

The fact that intake was usually normal when ingested food was confined to the stomach, but intake was much larger than normal when ingested food drained from the stomach during sham feeding, apparently confirmed the importance of gastric negative feedback for the control of meal size. The possibility that gastric negative feedback was due to abnormally high intragastric pressure in the cuff-closed stomach was rejected when the same results occurred in rats with the cuff closed that had an open gastric catheter to relieve excess pressure (13). Eating when the pyloric cuff was closed also did not appear to be aversive because intake did not change during 14 consecutive tests (23), and sham feeding was normal (6).

Although normal intake when the pyloric cuff was closed was interpreted as demonstrating the importance of gastric stimuli for the control of meal size, it is clear that orosensory stimuli of ingested food also have satiating effects (27). The relative contribution of oral and gastric satiating mechanisms to the control of meal size when the pyloric cuff is closed has been investigated only once to our knowledge. Davis et al. (6) reported that the first time rats ate 0.8 M sucrose with the pyloric cuff closed, and a chronic gastric cannula was open, meal size was twice as large as when the cuff and the cannula were closed. They concluded that gastric satiating mechanisms made a significant contribution to the control of meal size, perhaps as much as 50% under these conditions, but the orosensory stimuli of sham feeding were sufficient to terminate eating when ingested sucrose solution did not accumu-
late in the stomach. This confirmed an earlier report (19) of the satiating effect of orosensory stimuli during sham feeding after short periods of food deprivation.

When sham feeding was repeated for five trials by Davis et al. (6), 30-min intakes increased further to a maximum that was more than twice as large as the intake in the first sham feeding test. This further increase of intake with repetition was interpreted as extinction of conditioned, orosensory inhibitory control. Thus Davis et al. concluded that both orosensory and gastric stimuli produced significant negative-feedback control of intake when a rat ate with the pyloric cuff closed.

To avoid this synergism between orosensory and gastric negative feedbacks of ingested preloads, it is necessary to infuse liquids directly into the cuff-closed stomach. Phillips and Powley (21) used this method in recent experiments. They observed an inhibitory effect of intragastric preloads of carbohydrates or Isocal, a nutritionally complete liquid diet (Mead Johnson, Evansville, IN), that was accounted for by the volume of the preloads. In contrast to earlier claims of Deutsch and Gonzalez (10, 11), there was no evidence that the nutrient content of the preloads contributed to the inhibition of intake.

A major difference between the protocols of Deutsch and Gonzalez (10, 11) and Phillips and Powley (21) that might have explained the different results was that Deutsch and Gonzalez infused their gastric loads into the stomach during the initial part of the intake test, whereas Phillips and Powley infused their gastric loads just before the intake test.

To investigate the relevance of that difference in experimental design and to extend the search for conditions under which nutrient stimuli in the stomach could produce a satiating effect, we compared the inhibitory effects of 3, 6, or 12 ml of saline (0.15 M) or milk infused into the cuff-closed stomach during the first 6 min of a 30-min intake test after 19 h of food deprivation instead of 7 h as Phillips and Powley (21) used. We also evaluated the reproducibility of the effects of the infusions by giving each of them in five consecutive tests. By measuring intake at 3-min intervals and recording the rate and pattern of licking continuously, we were able to measure the effect of the volume or chemical content of these infusions on gastric negative-feedback control of intake and licking during the meal for the first time.

**MATERIALS AND METHODS**

**Methods**

Sprague-Dawley male rats, obtained from Taconic Farms (Germantown, NY), were housed in polycarbonate, wire-bottomed cages (24 cm × 20 cm × 29 cm) in an environment maintained at 23.5°C. The lighting schedule was a 12:12-h light-dark cycle; lights were on from 0700 to 1900. On weekdays, rats were maintained on a milk diet consisting of sweetened condensed milk (Borden Eagle Brand, Columbus, OH) diluted 1:2 with distilled water to which was added 3 ml of Poly-Vi-Sol vitamin drops (Mead Johnson)/900 ml water. On weekends, rats were given ground chow (Rodent Laboratory Chow-5001; Ralston Purina, St. Louis, MO). Rats always had access to tap water. Rats were adapted to their housing environment and maintenance diets for at least 2 wk.

Approximately 7–10 days before surgery, rats were deprived of food, but not of water, for 19 h (1500–1000) 5 days/wk and adapted to licking milk in testing cages. The test cages were the same as those used for housing except a special opening was cut out 7 cm above the floor for the attachment of a lickometer block (DiLog Instruments, Tallahassee, FL). The external side of the lick block held a 25-ml glass buret tube graduated to 0.1 ml (Kimax, Kimble Glass) that was fitted with a stainless steel sipper tube with Crazy Glue (Elmer’s Products, Columbus, OH). A moveable plastic bar was fastened ~5 cm from the end of the sipper tube to position the tip of the sipper tube 0.3 cm distal to the inner surface of the steel plate. After filling the buret with the milk diet, a size-00 stopper was used to plug the open end.

Each time the tongue of the rat made contact with the sipper tube, it completed a lickometer that passed no more than 60 μA through the animal. The time of each lick to the nozle and the millisecond was stored in computer memory. At the end of a test, these times were transferred to data files for later analysis (see Microstructural Analysis).

**Construction of the pyloric cuff and gastric catheter.** The construction of the pyloric cuff was modified from Young and Deutsch (29) and used Silastic sheeting (SF Medical Pharm Elast, Hudson, MA), Silastic tubing (Technical Products, Decatur, GA), and Silastic medical adhesive silicon type A (Dow-Corning, Midland, MI).

The construction of the gastric catheter was modified from that of Davis and Campbell (3) by use of 3.0-cm lengths of intramedic polyethylene tubing (PE-280; Clay Adams-Becton Dickinson, Parsippany, NJ), Marlex mesh (monofilament knitted polypropylene; C. R. Bard, Cranston, RI) cut into 2.0 cm × 2.0 cm squares, 20-cm lengths of Silastic tubing [inner diameter (ID) 0.078 in.; Technical Products, Decatur, GA], and Silastic medical adhesive silicon type A (Dow-Corning). One end of the polyethylene tubing was heat flared to form a round disk. A small hole was cut in the center of the square of Marlex mesh, and the polyethylene tubing was pulled through the mesh until the flared end was flat against the mesh. The Silastic tubing was then friction fitted over the free, unflared end of the polyethylene tubing and pushed down against the mesh. Silastic adhesive was placed around the junction of the tubing and the Marlex mesh and was allowed to dry overnight.

A sleeve of Silastic tubing was used to cover the exteriorized portions of the pyloric cuff and gastric catheter. This sleeve helped to prevent damage caused by rats grooming the site of exteriorization. For the cuff, a 4.0-cm segment of 0.078-in.-ID Silastic tubing was placed perpendicularly through the center of a 3.0 × 3.0 cm square of Marlex mesh. It was fixed in place 0.2 cm from the base of the tube with Silastic adhesive. The sleeve for the gastric catheter was prepared by use of the same procedure with a 4.0-cm segment of 0.132-in.-ID Silastic tubing. After construction, all sleeves were allowed to dry overnight.

**Surgery**

Rats weighed 250–325 g at the time of surgery. All rats were implanted with a pyloric cuff and a gastric catheter. Implantation of the pyloric cuff and gastric catheter was performed under surgical anesthesia with Chloropen, a mixture of pentobarbital (8.9 μg/ml) and chloral hydrate (42.5 μg/ml; 3 ml/kg ip). The pyloric region was exposed through a midline abdominal incision, and the pyloric sphincter was
separated from pancreatic and other tissue by blunt dissec-
tion. With the nonglue side of the pyloric cuff facing the
ventral surface of sphincter, the glue plug of one end of the
cuff was grasped with a hemostat and pulled under the
sphincter until it was centered under the dorsal surface of
the sphincter. The plugged ends of the cuff were then sutured
together using a mattress stitch of 3-0 silk; the trailing end of
tubing extended rostrally.

The cuff was inflated by infusing a volume of bacteriostatic
(0.9%) saline that was sufficient to occlude the pylorus. This
volume (0.35–0.50 ml) was recorded for each rat and was
used throughout testing.

In early experiments, verification of cuff placement at the
end of the experiment (see Verification of Cuff Placement)
revealed that the cuff had slipped down onto the proximal
duodenum in six rats. To prevent this, a band of Marlex mesh
(1.5 cm × 3.0 cm) was placed over the inflated cuff in subse-
cquent surgeries, and the proximal edge of the mesh was
sutured (4-0 silk) to the antrum and the distal edge was
sutured to the proximal duodenum. The band of mesh al-
lowed the cuff to be inflated, but it prevented the cuff from
slipping caudally.

Then the inflation tube of the cuff was pulled through a
hole in the abdominal wall, leaving enough slack to allow the
stomach to move freely, and then it was led subcutaneously
to emerge out on the posterior neck. A sleeve of Silastic
tubing with a base of silastically adhered Marlex mesh was
pushed down over the exteriorized end of the inflation tube.
The corners of the mesh were sutured (3-0 silk) to the under-
lying muscle to keep the exteriorized tube and sleeve in an
upright position. Silastic adhesive was applied between the
sleeve and the tubing to prevent slippage.

To implant the gastric catheter, a hole was made with a no.
11 scalpel blade along the greater curvature of the fundus
just distal to the limiting ridge, and a purse-string suture of
3-0 silk was placed around it but not tied. With the use of a
no. 10 scalpel blade, two small incisions were made in the
flared end of the catheter to make a slit about 0.5 cm × 0.1
cm. The slit of the flared end was slid into the hole of the
stomach and twisted counterclockwise. The purse-string su-
ture was then tied tight around the catheter. The square of
Marlex mesh glued to the base of the catheter was sutured
(3-0 silk) onto the stomach. The catheter was then exterior-
ized and fixed in the same way as the inflation tube of the
pyloric cuff. The gastric catheter was plugged with a segment
of Silastic tubing (0.04-in. ID) filled with dried Silastic adhe-
sive to prevent leakage of gastric contents.

Adaptation to Procedure

On the fifth and sixth postoperative days, the cuff of each
rat was inflated with one-half of the volume required to oc-
clude the pylorus. This took place during the normal test-
ing time in the home cage without access to food or water.
On the same day, the gastric tube was flushed with 5 ml of 0.9%
saline heated to 37°C. On day 7, the cuff was inflated with the
full volume. On day 8, when the cuff was fully inflated, each
rat was permitted to consume 5 ml of the milk diet. On day 9,
the rat was allowed to ingest 10 ml of the diet while the cuff
was fully inflated. Once a rat ate apparently normally under
these conditions, testing began.

Testing

Rats were deprived overnight of food but not water for
19 h. At 0945, water bottles were removed and each rat’s
pyloric cuff was inflated with the predetermined volume of
saline. Rats were transferred to lickometer cages, and the
exteriorized end of the gastric tube was connected to a 20-ml
syringe by Silastic tubing (0.040-in. ID).

At 1000, the rats were given access to milk, and gastric
infusions of 3, 6, or 12 ml of milk or 0.9% saline were
infused through the first 6 min at rates of 0.5, 1.0, or 2.0
ml/min. This resulted in total volumes of 3, 6, or 12 ml. In
baseline tests, an empty syringe was driven by the pump but
did not connect to the gastric tube. After the infusions
stopped, the rats continued to have access to milk for the
remaining 24 min of the 30-min test. Intake was measured every 3 min.

At the end of the test, the diet was removed, and 5 ml of
isotonic saline warmed to 37°C was infused through the
gastric catheter, the stomach contents were aspirated, and
the volume was measured by subtracting the 5 ml infused
from the volume aspirated. Then the pyloric cuff was deflated
by withdrawing the saline. The volume of saline withdrawn
from the cuff was compared with the volume infused into it to
confirm that leakage had not occurred during the test. Water
was returned 15 min after the test, and milk diet was re-
turned 30 min after the test.

The protocol consisted of six gastric infusions (3, 6, and 12
ml of milk and of saline) for 5 days. Each infusion of milk or
saline was preceded by 5 days of no infusion. The sequence of
volumes infused was not fixed and did not appear to affect the
results. Only one rat received all the infusions (Table 1). The
other 19 rats completed at least one sequence of 5 days of no
infusion and 5 days of milk or saline infusion. The reason
that rats failed to complete the protocol was a syndrome that
consisted of the acute onset of anorexia, abdominal disten-
tion, and weight loss. This could occur at any time after
surgery. Removal from testing, fluid supplements, sweet
milk diet or ground chow mixed with milk, and metoclopra-
dine (5 mg/ml; 0.2 ml im once or twice each day), a stimulant
of gastric motility and emptying, permitted rats to recover
fully and to return to experimental testing. Rats that failed to
respond to this therapy were euthanized. We wish to empha-
size that when recovery occurred, it was apparently complete
because rats regained the body weight that had been lost.

Table 1. Tests in individual rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>Milk</th>
<th>Saline</th>
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<tr>
<td></td>
<td>3</td>
<td>6</td>
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<td></td>
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Rats that failed to respond to this therapy were euthanized. We wish to empha-
size that when recovery occurred, it was apparently complete
because rats regained the body weight that had been lost.

Nos. at top (3, 6, and 12) indicate volume in milliliters; x indicates
that the rat received 5 days of that infusion preceded by 5 days
without that infusion.
Results of our study showed that normal intakes, as well as the effects of infusions on intake, were largely unchanged. Although the effects of mechanical obstruction at the site of the pyloric cuff were not observed, our results suggest that the cause of the syndrome is unknown. Phillips and Powley (21) reported a similar syndrome in rats with pyloric cuffs, but it was not observed in our previous experiments (20, 22, 24), and it was not reported by others (13, 14, 26). Although the syndrome did not occur in experiments that did not use sequential testing with the pyloric cuff closed, the relative importance of sequential testing to the syndrome remains to be demonstrated.

Verification of Cuff Placement

At the end of the experiment, the placement of the pyloric cuffs was verified using a procedure similar to that described by Phillips and Powley (21). All rats were anesthetized with Chloropent, and an abdominal incision was made to expose the stomach and duodenum. When the cuff was located, the distance between the proximal edge of the cuff and pyloric sphincter was measured. In 20 rats (Table 1), this distance was zero, indicating that the cuff had remained in place. In six other rats, however, the cuff had slipped 0.3–6.0 cm down onto the proximal duodenum. This occurred only in rats in which a band of Marlex mesh was not sutured over the cuff. The data from these rats were analyzed separately.

In addition to testing the placement of the cuff, we also verified that when the cuff was inflated, it did not leak and it prevented passage of gastric contents into the duodenum. We performed a microstructural analysis of the licking behavior of the rats to assess the impact of the infusions. The microstructural variables are obtained from an analysis of the intervals between successive licks (interlick intervals; ILIs). The licking behavior of rats ingesting liquids is characterized by bouts of licking at a high constant rate, separated by pauses of varying durations (5). We defined clusters of these bouts of licking as a run of licks with ILIs <500 ms. The number of licks within a cluster was defined as the size of the cluster. The number of licks and the average size and number of clusters were calculated separately for the first 6 min and last 24 min of the test to assess the effects of the infusion on the licking during the infusion and during the time after the infusion.

The data were analyzed by ANOVA calculated by Systat 7.0 for Windows, a statistical analysis software program. Statistical comparisons of rate of licking curves were done by repeated-measures analysis across the 1-min intervals and independent measures among the groups. The between-group analysis was necessary because of the absence of some data files. The conventional 0.05 probability level for rejection of the null hypotheses was used in all tests of significance.

The experiments were approved by the Institutional Animal Care and Use Committee of Weill Medical College of Cornell University.

Thirty-Minute Intakes

There was a significant effect of gastric infusions on 30-min intakes \( F(3,52) = 9.80, P = 0.0001 \), but there was no difference in the effects of milk or saline infusions \( F(1,52) = 0.17, P = 0.70 \) and no interaction between infusions and test days \( F(3,52) = 0.73, P = 0.54; \) Fig. 1]. The effect of infusions was entirely due to the inhibition of intake by the 12-ml infusions \( P < 0.05 \); smaller infusions did not change intake significantly compared with no infusion.

In six rats, the pyloric cuff slipped down onto the duodenum sometime during the experiments. The most complete data from these rats were from infusions of milk. Because ingested and infused milk could empty into a variable length of the upper duodenum in these rats, we compared their results with the six rats in which the cuff remained on the pylorus.

Analysis of the results showed that there was no significant effect of days of infusion \( F(4,169) = 1.43, P = 0.23 \) and no significant difference in the effect of the infusions between rats with cuffs on the duodenum and rats with cuffs on the pylorus \( F(1,43) = 2.87, P = 0.10; \) Fig. 2]. There was a significant effect of infusion in both groups of rats \( F(3,43) = 4.77, P = 0.006 \), but only the 12-ml infusions inhibited intake significantly compared with no infusion \( P < 0.05 \). Although the
12-ml infusions appeared to decrease intake more in rats with cuffs on the pylorus than in rats with cuffs on the duodenum, there was no significant interaction between cuff location and inhibition of intake \( F(3,43) = 0.50, P = 0.67 \). Although there was no interaction effect in this analysis, when the 12-ml infusions were compared separately in the analyses of 3-min intakes and microstructure (see below), the 12-ml infusions produce a significantly larger inhibition in rats with pyloric cuffs than in rats with duodenal cuffs (Fig. 2).

Three-Minute-Interval Intakes

The 3-min intakes confirmed and extended the results of the 30-min intakes in that 3 and 6 ml of saline or milk had little or no effect on intake compared with when no infusion was given, but 12-ml infusions did.

Three-milliliter infusions did not change intake significantly \( F(2,23) = 0.90, P = 0.42 \), but there was a significant effect of interval \( F(9,207) = 108.01, P = 0.0001 \) and a significant interaction between interval and kind of infusion \( F(18, 207) = 2.47, P = 0.001 \). Post hoc analysis of the interaction effect revealed that infusions of 3 ml of milk decreased intake significantly in the 6- to 9-min interval compared with infusions of 3 ml of saline but not compared with no infusion \( F(2,23) = 4.12, P = 0.03 \); Fig. 3. Intakes after infusions of 3 ml of saline were not significantly different from no infusion.

Six-milliliter infusions did not decrease intake significantly compared with no infusion \( F(2,23) = 1.56, P = 0.23 \); Fig. 4. There was a significant effect of interval \( F(9,207) = 63.75, P = 0.0001 \), but there was no significant interaction \( F(18,207) = 1.01, P = 0.45 \).

In contrast with the infusions of 3 or 6 ml, 12-ml infusions inhibited intakes significantly \( F(2,23) = 7.76, P = 0.003 \), but there was no significant difference between milk or saline infusions (Fig. 5). There was also a significant effect of interval \( F(9,207) = 25.56, P = 0.0001 \) and a significant interaction \( F(18,207) = 3.94, P = 0.0001 \). Post hoc analysis of the interaction revealed significant effects at 3, 6, and 9 min, but not at 12, 15, or 18 min. Because all of the intakes after 18 min were very small, they were not analyzed.
In the first 3 min, both infusions decreased intake compared with no infusion (Fig. 5). This gave a significant overall effect $F(2,23) = 4.17, P = 0.03$, but the difference was not significant by Tukey’s honestly significant different (HSD) tests.

From 3 to 6 min, the infusions decreased intake compared with no infusion. The difference was sufficient to produce a significant overall effect $F(2,23) = 12.11, P = 0.0003$ and a significant effect by Tukey’s HSD test ($P < 0.05$). Note that there was no significant difference between the effect of the milk or saline infusions.

From 6 to 9 min, there was an overall effect of infusion $F(2,23) = 3.94, P = 0.03$. Milk infusions decreased intake significantly compared with no infusion ($P < 0.05$), but saline infusions did not. There was no significant difference, however, between milk and saline infusions. Note that rats did not eat more later in the test when 12-ml infusions of milk or saline decreased intake during the first 9 min of the test (Fig. 5).

Three-Minute-Interval Intakes: Pyloric and Duodenal Cuffs

We also analyzed 3-min intakes during and after 12-ml infusions of milk in rats with cuffs on the pylorus and in rats with cuffs on the duodenum and compared these intakes to no infusion in the same rats (Fig. 6). We used the 12-ml infusions because they were the only infusions that produced decreased intakes compared with no infusion (Fig. 2).

There was a significant overall effect of infusion $F(2,19) = 5.68, P = 0.012$ and of interval $F(9,171) = 34.94, P = 0.0001$, and there was a significant interaction $F(18,171) = 3.45, P = 0.0001$. Infusions into...
rats with pyloric cuffs decreased intake significantly compared with no infusion \((P < 0.05)\), but infusions into rats with duodenal cuffs did not. Follow-up analysis of the interaction revealed significant effects in the 3- to 6-, 6- to 9-, and 9- to 12-min intervals.

In the 3- to 6-min interval, there was a significant effect of infusion \([F(2,19) = 8.26, P = 0.003]\). Infusions into rats with pyloric cuffs decreased intake compared with no infusion \((P < 0.05)\), but infusions into rats with duodenal cuffs did not.

There was also a significant effect of infusions in the 6- to 9-min interval \([F(2,19) = 6.86, P = 0.006]\) and in the 9- to 12-min interval \([F(2,19) = 4.23, P = 0.03]\). Infusions into rats with pyloric cuffs decreased intake significantly compared with no infusion in these intervals \((P < 0.05)\), but infusions into rats with duodenal cuffs did not.

### Gastric Volume Recovered

We measured the volume of gastric contents at the end of each test and compared it with the intake plus the volume infused (Table 2) to investigate the possibility that gastric volume at the end of the test terminated ingestion. The volumes recovered after milk or saline infusions were not significantly different \([F(1,19) = 0.91, P = 0.39]\). With both kinds of infusions, however, there was a significant effect of the volume infused \([F(3,33) = 7.27, P = 0.0007]\). The volumes recovered after infusions of 6 and 12 ml were significantly larger than recoveries after no infusions (Table 2, \(P < 0.05\)). They were not, however, different from recoveries after infusions of 3 ml, and they were not different from each other. Because the volumes recovered after infusions of 3, 6, and 12 ml were not significantly different and only the 12-ml infusions decreased 30-min intakes, differences in the volumes recovered were not correlated with the inhibition of intake by the infusions (compare Fig. 1 and Table 2).

Because the volume recovered equals volume ingested plus volume infused plus volume of salivary and gastric secretions, we could calculate the volume in the stomach that was not due to the volume ingested by subtracting the volume ingested from the volume recovered. These noningested volumes were not significantly different after the milk and saline infusions (Table 2; \(F(1,19) = 0.01\)). The noningested volumes were a function of the volume of infusions. All of the infusions produced larger noningested volumes than no infusion \([F(3,33) = 152.33, P = 0.0001]\), and they were all significantly different from each other \((P < 0.05)\). Note that the significant incremental differences among the noningested volumes at the end of the tests were not associated with incremental differences in the inhibition of intake.

By subtracting the volume infused from the noningested volumes, we obtained an estimate of the volume of endogenous salivary and gastric secretions under the four infusion conditions. The estimated volumes of secretions did not differ after milk or saline infusions \([F(1,19) = 0.28, P = 0.60]\) and did not differ across the volumes of infusions \([F(3,33) = 0.52, P = 0.67; \text{Table 2}]\). The lack of difference between milk and saline infusions on the volume of secretions was unexpected. It is fortuitous for the interpretation of the effect of the infusions because it eliminates the potential variable of differential volume of endogenous secretions produced by different volumes, rates, or kinds of infusions compared with intakes when no intragastric infusion was made.

### Microstructure of Licking

During the first 6 min of the tests with the 12-ml infusion, the number of licks made by the rats was significantly less than during the first 6 min on the baseline (no infusion) tests \([F(1,109) = 80.3, P < 0.001; \text{Fig. 7A}]\). This effect was independent of the type of infusion because there was no significant difference between the milk and saline infusions \([F(1,51) = 2.4, P = 0.126]\). The effect was consistent across the five infusion tests, since there was no significant difference

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**Table 2. Comparison of mean intakes and gastric volume recovered**

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<thead>
<tr>
<th></th>
<th>0 ml</th>
<th>3 ml</th>
<th>6 ml</th>
<th>12 ml</th>
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<tr>
<td><strong>Milk (n = 6-7)</strong></td>
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<tr>
<td>Intake, ml</td>
<td>15.0±0.87</td>
<td>11.9±0.88</td>
<td>11.1±0.90</td>
<td>4.7±0.65*</td>
</tr>
<tr>
<td>Recovered, ml</td>
<td>21.4±2.03</td>
<td>22.5±1.70</td>
<td>24.6±1.29*</td>
<td>22.7±1.21*</td>
</tr>
<tr>
<td>Noningested volume, ml</td>
<td>6.4±0.86</td>
<td>10.6±0.99*</td>
<td>13.5±1.05†‡</td>
<td>18.0±1.02‡</td>
</tr>
<tr>
<td>Infused, ml</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Secretions, ml</td>
<td>6.4±0.86</td>
<td>7.6±0.99</td>
<td>7.5±1.05</td>
<td>6.0±1.02</td>
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<td><strong>Saline (n = 6-7)</strong></td>
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<tr>
<td>Intake, ml</td>
<td>13.2±0.75</td>
<td>14.2±0.68</td>
<td>10.9±0.85</td>
<td>6.6±1.11*</td>
</tr>
<tr>
<td>Recovered, ml</td>
<td>20.3±0.35</td>
<td>22.3±0.80</td>
<td>24.4±1.29*</td>
<td>25.9±2.01*</td>
</tr>
<tr>
<td>Noningested volume, ml</td>
<td>7.2±0.50</td>
<td>8.2±0.91*</td>
<td>13.5±0.47†‡</td>
<td>19.2±1.28‡‡</td>
</tr>
<tr>
<td>Infused, ml</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Secretions, ml</td>
<td>7.2±0.50</td>
<td>6.5±0.91</td>
<td>7.5±0.47</td>
<td>7.2±1.28</td>
</tr>
</tbody>
</table>

Data are means ± SE measured after no infusion (0 ml) or infusion of 3, 6, or 12 ml during the first 6 minutes of 30-minute tests. Recovered refers to the volume of gastric contents aspirated at the end of the tests. Noningested volume was calculated by subtracting the volume of intake from the volume recovered. Secretions were calculated by subtracting the volume infused from the noningested volume. *Significantly different from the volume after no infusion, \(P < 0.05\). †Significantly different from the volume after the infusion of 3 ml, \(P < 0.05\). ‡Significantly different from the volume after infusion of 3 or 6 ml, \(P < 0.05\).
rats had received only 2 ml of either saline or milk in addition to that ingested voluntarily. This additional 2 ml reduced the number of licks during the first minute of the tests significantly from an average of 229.0 ± 17.1 licks in the baseline tests to an average of 127.6 ± 13.5 licks in the infusion tests \( F(1,107) = 21.7, P < 0.001 \). There was no effect, however, due to the type of infusion \( F(1,107) = 2.0, P = 0.161 \). This reduction in the number of licks was due solely to a significant reduction in the average size of the clusters from 53.7 ± 6.9 licks in the baseline tests to 23.7 ± 3.2 licks in the infusion tests \( F(1,104) = 14.9, P < 0.001 \). There was no significant difference in the number of clusters between the baseline (5.7 ± 0.53) and infusion (5.6 ± 0.45) tests \( F(1,107) = 0.05 \).

The 6-ml infusions decreased the number of licks significantly during the first 6 min relative to the same period during baseline tests \( F(1,120) = 13.4, P < 0.001; \) Fig. 8A. The decrease in the number of licks by infusions did not change significantly across the five infusion tests \( F(4,56) = 0.5 \).

The smaller number of licks was entirely due to a significantly smaller number of clusters \( F(1,120) = 13.5, P < 0.001; \) Fig. 8C because the size of clusters did not change \( F(1,118) = 0.2; \) Fig. 8B. The type of infusion did not affect the reduction in the number of licks \( F(1,159) = 0.9 \) or the reduction in the number of clusters \( F(1,159) = 2.4, P = 0.123 \).

This inhibitory effect of the infusions could be detected as early as the first minute of the tests when the

Figure 7. Average number of licks, cluster size, and number of clusters during the first 6 min (A–C) and last 24 min (D–F) of the tests. Variance is SE. Open bars are baseline (no infusion) tests, and solid bars are infusion tests; \( n = 7 \) for saline infusions and \( n = 6 \) for milk infusions. *Significantly different from baseline, \( P < 0.001 \). #Significantly different from baseline tests for milk infusion, \( P < 0.05 \).

The effect of the 12-ml infusion on licking during the first 6 min of the tests persisted for the remainder of the test because there were fewer licks during the last 24 min of those tests than during the same period on the baseline tests \( F(1,119) = 22.4, P < 0.001; \) Fig. 7D. The effect on the number of licks was independent of the type of infusion because there was no significant difference between them \( F(1,51) = 0.001 \). It was also consistent across the five infusion tests, since there was no significant difference in this measure across those five tests \( F(4,48) = 1.8, P = 0.149 \). The decrease in the number of licks was due to a significant reduction in the size \( F(1.82) = 9.7, P = 0.001; \) Fig. 7E and number of clusters \( F(1,119) = 8.9, P < 0.001; \) Fig. 7F. Although saline infusions decreased the number of clusters significantly compared with no infusion \( P < 0.05 \) and milk infusions did not (Fig. 7F), this apparent difference appears to be due primarily to the smaller number of clusters in the baseline experiments before milk infusions compared with baseline experiments before saline infusions.

This inhibitory effect of the infusions could be detected as early as the first minute of the tests when the
During the last 24 min of the tests, 6-ml infusions of milk decreased the number of licks significantly [F(1,61) = 14.1, P < 0.001], but 6-ml infusions of saline did not [F(1,57) = 0.03; Fig. 8D]. Furthermore, the difference in the number of licks during the last 24 min between the milk and saline infusion groups was statistically significant [F(1,59) = 9.2, P = 0.004]. This reduction in the number of licks produced by milk infusions was maintained over the five infusion tests, since there was no significant difference in this measure over those tests [F(4,26) = 1.5, P = 0.24]. This decreased number of licks was due solely to a significant reduction in the number of clusters [F(1,61) = 12.7, P = 0.001; Fig. 8F] because cluster size did not change [F(1,53) = 0.9; Fig. 8E].

In the tests in which the rats received 3-ml infusions of milk and saline, the number of licks during the 6-min infusion was not significantly different from the number of licks during the same period in the baseline tests [F(1,113) = 0.2; Fig. 9A]. During the last 24 min of the tests, however, the rats made significantly fewer licks after milk infusions than they had in their baseline tests [F(1,48) = 5.7, P = 0.021; Fig. 9D]. In this case, however, there was significant variation in this measure across the five infusion tests [F(4,20) = 3.9, P = 0.018] because of the fact that the number of licks increased linearly [F(1,20) = 6.8, P = 0.017] across the five tests. By the last milk infusion test, the number of licks during the last 24 min of the test (367.0 ± 71.0 licks) was virtually the same as the baseline average (357.7 ± 55.8 licks).

The number of licks during the first 6 min in the groups receiving the 3-ml infusions of milk or saline was not different [F(1,56) = 1.2, P = 0.313; Fig. 9A], but there was a significant difference during the last 24 min [F(1,56) = 6.7, P = 0.012; Fig. 9D]. This difference was due to individual differences in assignment of animals to the experimental groups, because this difference appeared in the baseline tests as well [F(1,56) = 6.7, P = 0.012]. Note that neither kind of infusion produced a significant difference in cluster size or number from its respective baseline in either the first 6 or last 24 min.

We compared the intakes and licking behavior of the rats with cuffs on the duodenum to that of rats that had cuffs on the pylorus during tests in which no infusion was given or 12 ml of milk was infused in the first 6 min. There was no significant difference among the five baseline tests in volume ingested for the rats with duodenal cuffs [F(4,16) = 0.8] or those with the pyloric cuffs [F(4,23) = 0.3], so the data were collapsed over these tests in this analysis. Rats with duodenal cuffs, however, ingested significantly more during the baseline tests than rats with pyloric cuffs [17.5 ± 1.2 vs. 11.5 ± 1.1 ml; F(1,47) = 13.1, P = 0.001]. This was due entirely to significantly more clusters [66.2 ± 7.4 vs. 40.5 ± 6.3; F(1,47) = 7.0, P = 0.011] because cluster size was not significantly different [43.4 ± 5.7 vs. 35.9 ± 4.9 licks; F(1,47) = 1.0].

In the 12-ml infusion tests, there was no significant difference across the five tests among the rats with pyloric cuffs [F(4,22) = 1.6, P = 0.198] or duodenal cuffs [F(4,16) = 0.5], so the data from the five tests with the two groups were pooled for statistical analysis. In these tests, the rats with the duodenal cuffs ingested significantly more [11.9 ± 1.2 vs. 5.0 ± 1.1 ml; F(1,46) = 17.0, P < 0.001] because of significantly more clusters [51.6 ± 7.6 vs. 21.6 ± 6.7; F(1,46) = 8.7, P = 0.005] rather than larger ones [30.9 ± 3.1 vs. 25.8 ± 4.3; F(1,43) = 0.6].

During the first 6 min of the baseline tests, rats with the duodenal cuffs had significantly more licks than rats with pyloric cuffs [t(47) = 2.52, P = 0.02; Fig. 10A]. This was due to the joint effect of slightly larger and slightly more clusters (Fig. 10, B and C); these differences, however, were not statistically significant [cluster size, t(47) = 1.17, P = 0.25; cluster number, t(47) = 1.20, P = 0.24].

The infusion of 12 ml of milk in the first 6 min decreased the number of licks compared with baseline in rats with duodenal cuffs [t(38) = 3.45, P = 0.001] and in those with pyloric cuffs [t(52) = 4.81, P = 0.01; Fig. 10A]. In addition, rats with pyloric cuffs had a significantly smaller number of licks than those with the duodenal cuffs [t(43) = 3.24, P = 0.002; Fig. 10A]. This correlated with the smaller intake of the rats with pyloric cuffs during this interval.

The decreased number of licks produced by the infusion of milk in rats with duodenal cuffs was due to
In the 24 min after the milk infusion, the number of licks compared with baseline decreased significantly in rats with duodenal cuffs \( t(38) = 2.18, P = 0.04 \) and in rats with pyloric cuffs \( t(52) = 2.62, P = 0.01 \); Fig. 10D). Furthermore, the number of licks in rats with pyloric cuffs was significantly smaller than in the rats with duodenal cuffs \( t(43) = 2.63, P = 0.01 \). The decrease in the number of licks in rats with duodenal cuffs compared with baseline was due to a significant decrease in the size of clusters \( t(36) = 2.25, P = 0.03 \); Fig. 10E; the number of clusters did not change significantly \( t(38) = 0.37, P = 0.72 \); Fig. 10F). A similar pattern was also seen in rats with pyloric cuffs: cluster size almost decreased significantly \( t(37) = 1.95, P = 0.059 \), but cluster number did not \( t(52) = 1.40, P = 0.17 \). The rats with the pyloric cuffs had a significantly smaller number of clusters than those with duodenal cuffs \( t(43) = 2.73, P = 0.01 \), but there was no difference in cluster size \( t(26) = 0.43, P = 0.67 \).

**DISCUSSION**

The effect of the infusions depended on the kind of measurement. We used five measures of ingestion: 30-min intakes, 3-min intakes, number of licks, cluster size, and cluster number. The microstructural measures detected effects of infusions that the measures of intake did not.

**Thirty-Minute Intakes**

Twelve-milliliter infusions of saline or milk decreased 30-min intakes significantly, but 6-ml and 3-ml infusions did not (Fig. 1). The effect of the 12-ml infusions was not different across the five consecutive tests. This suggests that the effect was not aversive and that neither a conditioned aversion nor a conditioned satiation appeared during the repeated tests.

There was no significant difference in the inhibitory effect on intake between saline and milk infusions. This suggests that the volume of the infusion is the adequate gastric stimulus for the inhibition of 30-min intake. That conclusion is consistent with the results of Phillips and Powley (21) obtained under different experimental conditions, when infusions were made before the test meal.

The results of the 12-ml infusions of milk into the rats with duodenal cuffs are relevant to the suggestion by Phillips and Powley (21) that the inhibitory effects of gastric infusions attributed to fat by Deutsch and Gonzalez (10, 11) were due to placement of the cuff on the duodenum instead of the pylorus (29). Under our conditions, however, 12 ml of milk infused into rats with duodenal cuffs did not decrease intake more than 12 ml of milk infused into rats with pyloric cuffs; in fact, it decreased intake significantly less in the rats with duodenal cuffs (Fig. 2). It is possible, of course, that less duodenum was exposed to the gastric infusion in our experiments than in those of Deutsch and Gonzalez (10, 11). Further experiments are required to settle this point.
It is remarkable that rats continue to eat ~50% of their 30-min intakes in the 24 min after the saline infusions end (50% after 3 ml, 52% after 6 ml, and 50% after 12 ml; see Table 3 for 6-min intakes and Table 2 for total intakes). Note that this substantial intake occurs despite a calculated stomach volume at 6 min that is almost equivalent to (10.1 ml after 3 ml and 11.7 ml after 6 ml; Table 3) or exceeds (15.3 ml after 12 ml; Table 3) the total intake of 13.2 ml in 30 min after no infusion (Table 2). Furthermore, the intakes after the infusions occur despite gastric volumes that are much larger than the gastric volume at 6 min, when no infusion was given (63% larger after 3 ml, 58% after 6 ml, and 107% after 12 ml; Table 3).

The relatively weak potency of gastric negative feedback produced by the infusions can be measured as the change in milliliter ingested from when no infusion was given per milliliter of infusion (Table 4). The mean decrease of 30-min intakes by all infusions was less than the volume infused (range = 0.30–0.84 ml/ml) except for 6-ml infusions of milk. The mean value of 1.0 ml/ml for the infusions of 6 ml was mainly due to one rat that decreased 2.9 ml/ml infused. The mean of the other six rats was 0.68 ml/ml infused. With meals of ~5 ml and infusions of 2.5, 5.0, 7.5, and 10.0 ml, Phillips and Powley (21) reported a similar result of 0.50 ml/ml infused with considerable variance for infusions of <10 ml (see Fig. 1 of Ref. 21).

The effects of the infusions on intake were not only weak, but they were also variable. The coefficients of variation ranged from 28.8 to 234.2%, with median = 71%. The variance was such that there was no significant difference between the effects of milk and saline infusions [F(1,10) = 2.13, P = 0.18], no difference among the volumes of infusions [F(2,20) = 1.67, P = 0.21], and no interaction [F(2,20) = 1.15, P = 0.34].

The inhibitory effect of the 12-ml infusions could also have been due to rate of infusion rather than volume, because volume and rate of infusion were confounded in these experiments. The possible importance of rate is supported by the fact that 12-ml infusions significantly inhibited licking in the first minute after the infusion began (see RESULTS) and intake within the first 3–6 min (Fig. 5). Note that the same rapid rate of infusion did not decrease intake significantly in rats with duodenal cuffs. This suggests that the larger volume of gastrointestinal tract or some effect of duodenal stimulation reduced the effect of the rate of infusion. Our results do not decide between these two explanations.

Although 30-min intakes (meal size) did not reveal an effect of the infusions due to nutrient content, there are many examples of different patterns of ingestion during a meal producing the same intake [see Davis (2) for review; Refs. 16 and 17]. Thus we made four different measures of the pattern of ingestion during the meal to search for possible differences between saline and milk infusions during and after the infusions.

### Three-Minute-Interval Intakes

Three-minute-interval intakes during the infusions showed a significant and equivalent inhibition by 12 ml of saline and milk and no significant effect of smaller infusions (Table 5 and Figs. 3–5). After the infusions, however, 3- and 12-ml infusions of milk decreased intake during the 6- to 9-min interval, but saline infusions did not (Table 5). This is the first evidence in our experiments

### Table 3. Intakes and estimated gastric volumes

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Interval, min</th>
<th>Ingested, ml</th>
<th>Infused, ml</th>
<th>Gastric Volume, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0–3</td>
<td>3.25 ± 0.31</td>
<td>0</td>
<td>3.25 ± 0.31</td>
</tr>
<tr>
<td>3 ml</td>
<td>3–6</td>
<td>4.60 ± 0.60</td>
<td>1.5</td>
<td>6.10 ± 0.60</td>
</tr>
<tr>
<td>None</td>
<td>0–6</td>
<td>5.38 ± 0.80</td>
<td>0</td>
<td>5.38 ± 0.80</td>
</tr>
<tr>
<td>6 ml</td>
<td>0–3</td>
<td>6.31 ± 0.38</td>
<td>0</td>
<td>6.31 ± 0.38</td>
</tr>
<tr>
<td>None</td>
<td>0–6</td>
<td>6.70 ± 0.93</td>
<td>3</td>
<td>10.70 ± 0.93</td>
</tr>
<tr>
<td>6 ml</td>
<td>0–3</td>
<td>6.31 ± 0.38</td>
<td>0</td>
<td>6.31 ± 0.38</td>
</tr>
<tr>
<td>None</td>
<td>3–6</td>
<td>4.31 ± 0.70</td>
<td>3</td>
<td>7.31 ± 0.70</td>
</tr>
<tr>
<td>6 ml</td>
<td>3–6</td>
<td>5.15 ± 0.33</td>
<td>0</td>
<td>5.15 ± 0.33</td>
</tr>
<tr>
<td>None</td>
<td>0–6</td>
<td>9.53 ± 1.00</td>
<td>6</td>
<td>15.03 ± 1.00</td>
</tr>
<tr>
<td>12 ml</td>
<td>3–6</td>
<td>3.46 ± 0.53</td>
<td>0</td>
<td>3.46 ± 0.53</td>
</tr>
<tr>
<td>None</td>
<td>0–6</td>
<td>7.13 ± 1.07</td>
<td>0</td>
<td>7.13 ± 1.07</td>
</tr>
<tr>
<td>12 ml</td>
<td>0–6</td>
<td>2.80 ± 0.72</td>
<td>12</td>
<td>14.80 ± 0.72</td>
</tr>
</tbody>
</table>

Ingested and volume data are means ± SE. Interval is minutes of test after no infusion (none) or infusions of 3, 6, or 12 ml of milk during the first 6 min. The gastric volumes were estimated on the assumptions that the volumes of ingested and infused milk were constant in the stomach during the first 6 min and that the volume of other gastric contents did not differ among the 3 infusions or no infusions (see Table 2).

### Table 4. Ratio of volume of milk ingested per volume of saline or milk infused

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Interval, min</th>
<th>Ratio of Ingested to Infused, ml/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 ml</td>
<td>0–6</td>
<td>0.61 ± 0.13</td>
</tr>
<tr>
<td>6 ml</td>
<td>0–3</td>
<td>0.40 ± 0.34</td>
</tr>
<tr>
<td>12 ml</td>
<td>0–6</td>
<td>0.14 ± 0.13</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 ml</td>
<td>0–6</td>
<td>0.22 ± 0.28</td>
</tr>
<tr>
<td>6 ml</td>
<td>0–6</td>
<td>0.46 ± 0.54</td>
</tr>
<tr>
<td>12 ml</td>
<td>0–6</td>
<td>0.38 ± 0.06</td>
</tr>
</tbody>
</table>

Interval is minutes of the test. Ratios of ingested to infused are means ± SE. A negative ratio denotes the decrease in volume ingested by the volume infused during the first 6 or entire 30 min of the test.
that nutrient stimuli in the stomach produced inhibitory effects on intake. Because these effects were so transitory, their biological significance is not clear.

In contrast to the results in pyloric cuff rats, there was no significant effect on 3-min intakes during or after infusion of 12 ml of milk into rats with duodenal cuffs compared with no infusions (Table 5 and Fig. 6).

**Number of Licks**

Infusions of 12 ml of milk and saline decreased the number of licks during and after the infusions (Table 5 and Fig. 7), and there was no significant difference between the effects of the milk and saline infusions. This is consistent with the results of 30-min intakes. Although the 12-ml infusions of milk in rats with duodenal cuffs decreased the number of licks significantly during and after the infusion (Table 5), the decrease was significantly less than the decrease produced by the same infusions into rats with pyloric cuffs (Fig. 10).

Infusions of 6 ml of milk and saline decreased the number of licks, although they had no significant effect on 3-min or 30-min intakes. Milk and saline infusions decreased the number of licks significantly and equivalently during the infusions (Table 5 and Fig. 8), but after the infusions, milk infusions decreased the number of licks significantly although saline infusions did not (Table 5 and Fig. 8). Thus the number of licks detected a significant effect of 6-ml infusions of milk that could not be accounted for by the volume or rate of the infusion. Note that this significant decrease in the number of licks was not sufficiently large to affect 3-min or 30-min intakes (Table 5).

Three-milliliter infusions produced only one significant decrease in the number of licks. That was after infusions of milk, but not after infusions of saline (Table 5 and Fig. 9).

### Cluster Size and Number

Changes in the number of licks during an interval can be due to a change in cluster size, cluster number, or both. The 12-ml infusions decreased cluster size and number during and after the infusions (Table 5 and Fig. 7). The only difference between saline and milk infusions was that saline infusions decreased the number of clusters after the infusions significantly compared with baseline, but milk infusions did not.

In contrast to the 12-ml infusions, 6-ml infusions decreased the number of licks by decreasing cluster number without changing cluster size (Table 5 and Fig. 8). Both saline and milk infusions decreased cluster number during the infusions. After the infusions, however, there was a differential effect: milk infusions decreased cluster number, but saline infusions did not.

Although 3-ml infusions of milk, but not saline, decreased the number of licks after the infusions, neither cluster size nor cluster number changed significantly (Table 5 and Fig. 9).

The microstructural effects of 12-ml infusions of milk in rats with duodenal cuffs were different from the effects in rats with pyloric cuffs. The decreased number of licks during infusions into rats with duodenal cuffs was not accompanied by significant changes in cluster size or number, but the same infusions decreased cluster size and number in rats with pyloric cuffs (Table 5 and Fig. 10). In the 24 min after the infusion, cluster size decreased in both groups of rats, but the number of clusters did not change (Table 5).

In summary, all measures detected a significant inhibitory effect of 12-ml infusions. During the infusions, there was no difference between infusions of saline or milk. After the infusions, two differences between milk and saline were found. First, milk infusions decreased intake in the first 3 min after the infusion, but saline infusions did not (Fig. 5). Second, saline infusions decreased the number of clusters after the infusion, but milk infusions did not (Fig. 7). Because these differential effects were small, we conclude that the inhibitory effect on 30-min intakes (meal size) of 12-ml infusions of milk and saline is due to gastric volume or rate of infusion but not to nutrient content. This conclusion is reinforced by the fact that the saline infusions diluted the nutrient content in the stomach, but the milk infusions did not.

If rate of infusion is the critical variable, the rate of entry into the stomach of ingested and infused liquid in these experiments (>2 ml/min, Table 3) is never attained during normal feeding in our experience. Thus either the rapid rate or the large volume required to inhibit intake in these experiments undermines the physiological relevance of gastric negative-feedback control of intake under our experimental conditions.

Although the results of the 12-ml infusions suggest that the stomach cannot detect the difference in nutrient content during and after infusions of saline or milk, the results of the 6-ml and 3-ml infusions provide some evidence for gastric detection of chemical content in addition to volume. After the 6-ml infusions, milk in-

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**Table 5. Summary of effects during and after infusions**

<table>
<thead>
<tr>
<th>Cluster Size and Number</th>
<th>During Infusion</th>
<th>After Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 ml</td>
<td>6 ml</td>
</tr>
<tr>
<td>3-min Intakes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Milk</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Milk, duodenal cuff</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>No. of licks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>NC</td>
<td>↓</td>
</tr>
<tr>
<td>Milk</td>
<td>NC</td>
<td>↓</td>
</tr>
<tr>
<td>Milk, duodenal cuff</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Cluster no.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>NC</td>
<td>↓</td>
</tr>
<tr>
<td>Milk</td>
<td>NC</td>
<td>↓</td>
</tr>
<tr>
<td>Milk, duodenal cuff</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

NC, no change; ↓, significant decrease compared with measures from tests in which no infusion was given.
fusions decreased the number of licks and the number of clusters, but saline infusions did not (Table 5 and Fig. 8). After the 3-ml infusions, milk infusions decreased the number of licks, but saline infusions did not (Table 5 and Fig. 9).

These differential effects are consistent with gastric detection of a nonvolumetric stimulus of milk infusions because stimulation of the small intestine was excluded. Note that these differential effects occurred after infusions, not during infusions. This makes it less likely that they are related to the rate of infusion. More experiments are required to define what stimulus of the milk infusions is being detected, i.e., osmotic, textural, or nutrient.

The differential effects of milk and saline on the number and microstructure of licks are important for three reasons. First, they demonstrated that the stomach detects some nonvolumetric aspect of milk in the absence of nutrient stimulation of the small intestine. Second, because this nonvolumetric information changes the number of licks and the number of clusters, but not the size of clusters, it is used to decrease the probability of reinitiating licking once it has stopped and is not used to change the number of licks emitted once a cluster of licks has begun (1). Third, this nonvolumetric information does not affect the control of the volume ingested during the meal as measured by 3-min intakes or the size of the meal measured by 30-min intakes. The failure of changes in the rate of ingestion to affect total intake is consistent with the recent reports of Kaplan and coworkers (16, 17). We interpret this failure to mean that the nonvolumetric information did not contribute to the negative feedback from the stomach that is integrated with the positive and negative feedbacks from the mouth to control ingestion during the meal under these conditions. It remains to be seen whether other conditions can be found where gastric negative feedback produced by nonvolumetric stimuli can decrease meal size.

The necessity of large infusions (12 ml) to inhibit meal size in these experiments is consistent with the earlier reports of Davis and coworkers (4, 7). They showed that confining ingested solutions of sucrose and salt in the stomach by closing a pyloric cuff decreased meal size only when intakes with the cuff open were large, ~20 ml.

That a large volume must be infused to inhibit intake significantly demonstrates that negative feedback due to gastric volume is not a potent control of meal size under these conditions. It has been suggested that the negative-feedback potency of gastric feedback is low in experiments with closed pyloric cuffs because the pyloric cuff prevents stimulation of the small intestine by ingested food that normally enhances the potency of the effects of gastric volume by decreasing gastric emptying through neural and hormonal mechanisms (25). Although such amplification of the response of gastric vagal afferent nerves to gastric volume has been demonstrated (25), this phenomenon has not been reported in the control of intake. Furthermore, when gastric contents emptied into the proximal duodenum in rats with duodenal cuffs, the inhibitory potency of the 12-ml infusions of milk on 3-min and 30-min intakes was reduced, not increased. The same result was obtained when Phillips and Powley (21) permitted their gastric preloads to empty from the stomach into the small intestine.

Another potential explanation for the low potency of the gastric negative feedback on intake is that intra-gastric infusions might not be as effectively monitored by the stomach as ingested liquids. This possibility requires demonstration in experiments designed to measure the quantitative contribution of pregastric and gastric negative-feedback effects of the ingested liquids. Such an experiment has not been done.

To our knowledge, these experiments are the first to measure the inhibitory effect of intra-gastric infusions on intake and microstructure during a meal in the rat. They demonstrate that when ingested milk and infused milk or saline are confined to the stomach, the gastric negative-feedback control of ingestion during the meal depends on the volume or rate of the infusion, but not on its nutrient content. The sensitivity of the stomach to volume or rate is relatively low because infusing ~50% of the volume of a meal (6 ml) during the first 6 min of eating under these conditions did not reduce meal size significantly.

Although there were only small and transient differential effects of 12-ml infusions of saline or milk, infusions of 3 and 6 ml of milk had larger and longer effects on the microstructure of licking than isovolumetric infusions of saline. This demonstrates that when ingested and infused liquids are confined to the stomach, the stomach detects some nonvolumetric information, probably nutrient, that decreases the rate of licking and the probability of reinitiating licking without changing the size of the meal or 3-min intakes during the meal.

Perspectives

The results of these experiments underline the importance of measuring intakes and the microstructure of licking during a meal to characterize the effect of a specific experimental manipulation of satiation. The advantages are specificity, quantitation, and temporal course of the effects. For example, the results show that the equal inhibitory effect of 12-ml infusions of saline and milk on intake occurred primarily during the infusions, whereas the nonvolumetric effects of infusions of 3 and 6 ml of milk on the rate and pattern of licking occurred after the infusions. The different temporal domains of these effects can be exploited to analyze the underlying mechanisms. Because the pyloric cuff confined infusions to the stomach, the site of their effects must be initiated by gastric afferent mechanisms.

The experiments also emphasize two important gaps in our knowledge about satiation. One is how meal size is maintained despite changes in the microstructure of licking. The other is the lack of information about the satiating effect of orosensory stimulation, especially its
interaction with gastric negative feedback. Until these two gaps are closed, we will lack an adequate physiological explanation of satiation and the role of gastric negative feedback in it.

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