Chemical specificities and intestinal distributions of nutrient-driven satiety

J. H. MEYER, M. HLINKA, Y. TABRIZI, N. DiMASO, AND H. E. RAYBOULD

Departments of Physiology and Medicine, University of California Los Angeles School of Medicine and the West Los Angeles and Sepulveda Veterans Affairs Medical Centers, Los Angeles, California 90073

Meyer, J. H., M. Hlinka, Y. Tabrizi, N. DiMaso, and H. E. Raybould. Chemical specificities and intestinal distributions of nutrient-driven satiety. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1293–R1307, 1998.—We measured intakes of sham- and naturally feeding rats during gut perfusions of nutrients. Our objectives were to determine 1) which nutrient products in gut lumen suppressed intakes; 2) how suppression by various nutrients is distributed along gut; and 3) whether time courses of suppression were similar among different nutrients. We found that satiating nutrients consisted of fatty acids only longer than 10 carbons, of monomeric carbohydrates only with affinity for the glucose transporter, and, among several amino acids, of only phenylalanine and tryptophan. Dimeric maltose had about the same potency as an isocaloric mixture of longer glucose polymers; since responses to either were blocked by a glucosidase inhibitor, each probably acted after hydrolysis to free glucose. Effective nutrients suppressed intakes about equally on infusion into duodenum vs. midgut, and the same nutrients also suppressed intakes when infused into colon. Food intakes were suppressed only while maltose was infused, not after it was stopped, but suppression persisted for 2 h after stopping perfusions with fatty or amino acids.

Like monkeys (24) and humans (17, 32, 34), rats can accurately sense dietary calories promptly after they enter the stomach. For example, these animals will reduce their caloric consumptions to compensate consistently for calories instilled into the stomach just before feeding (2, 27). Even when daily feedings are restricted to 4 h, rats are able to increase their intakes of food manyfold to compensate almost completely for caloric dilutions of the diet (1, 3). This drive to markedly increase the intake of diluted food over a foreshortened period of feeding implies that the animals can sense the caloric density of the diet as they consume it. In this and companion papers (27, 28), we define and use the term satiety as this ability to sense infused or ingested calories and to adjust food intakes in response.

There is ample evidence from intestinally perfused rats (22, 31), rabbits (36), and monkeys (12) that nutrients in small intestine powerfully inhibit natural or sham feeding in a dose-related fashion. Several studies suggest that nutrients signal such inhibition from ileum as well as duodenum and, in fact, that feedback from ileum might be even stronger than from more proximal bowel (13, 20). In our own prior experiments in dogs (9, 26), abnormally exposing ileum (in addition to jejunum) to digested food sharply reduced natural feeding in comparison with food intakes after the more usual confinement of nutrients to proximal small bowel. Thus either the ileum more potently signaled a suppression of intake than did jejunum or the magnitude of intestinal satiety varied with the total length of contact of digestive products.

The last considerations led us to postulate that nutrient-driven suppressions of food intakes might arise by recruitment of sensors along increasing intestinal lengths as caloric consumption varies. Many nutrients empty from the stomach at rates that vary initially with the ingested amount of each (27). As a result, the length of spread of nutrient digestive products along small intestine might also vary with amounts ingested. Variations of length of contact with changing rates of gastric emptying, in turn proportional to changing gastric loads, could give minute-to-minute information on quantities of macronutrients present in the stomach, even as calories are being consumed and even before caloric nutrients have completely emptied from the stomach.

We have examined this complex hypothesis in experiments on rats reported in this and two companion papers (27, 28). Our aims in this first study were threefold: 1) to determine whether intestinal satiety is induced by all products from the three classes of macronutrients (fat, carbohydrate, protein) or whether potency was confined to specific chemical structures in each class; 2) to establish whether and to what extent intestinal satieties could be induced by each class of macronutrient from distal and proximal bowel; and 3) to determine whether onset and persistence of suppression during and after intestinal infusions of nutrients varied among the three classes of macronutrients.

The following two basic, chronic rat preparations were used: 1) a modified sham-feeding model and 2) an intestinally perfused, naturally feeding model. In both preparations, nutrient perfusates entered small intestine via a catheter chronically implanted in the duodenum or via a catheter implanted at mid small intestine. Nutrients were infused in a range of doses that encompassed threshold and maximal responses. The potencies of satiety mechanisms in the proximal vs. the distal small intestine were compared by examining the steep-
ness of the dose response with the proximal vs. midgut infusions.

METHODS

Surgical Preparations

After anesthesia with pentobarbital sodium (50 mg/kg ip), fasted, 300-g male Sprague-Dawley rats were prepared for sham-feeding experiments as follows. The abdomen was opened, and a stainless steel cannula was placed in the most dependent part of the stomach along the ventral surface. The inner flange of the cannula was inserted with pressure through the small hole in the stomach wall, and then the cannula was buttressed with a purse-string suture around the perimeter. A pierced piece of Marlex mesh (to induce fibrotic adhesions) slid over and along the shaft of the cannula down to the serosal surface of the sutured stomach. Next, perfusion catheters were inserted into the proximal duodenum (≥2 cm from the pylorus) and at midgut (55 cm from the pylorus). The distal end of each perfusion catheter of PE-90 tubing was flared under flame and then inserted into a point incision through the bowel wall. A six-point, purse-string suturing (6-0 silk, cardiovascular, Ethicon) was made to close the entrance hole and buttress the perfusion catheter. The buttressing was reinforced by sliding a small patch of Marlex mesh over the catheter against the serosal surface of the bowel, where the Marlex was then held in place by a bushing of Tygon tubing that slid, in turn, over the PE-90 tubing against the Marlex. The Marlex was not sutured to duodenum but was sutured to the midgut with one 5-0 silk stitch on either side of the PE-90 tubing. The Marlex ensured sufficient fibrosis to prevent leaks and to anchor the PE-90 tubing. At the proximal end of the perfusion catheters in the neck, the 2 guideshaft of a no. 20 hypodermic needle (hub removed) was inserted into the PE-90 tubing to buttress the tubing against wear or ripping by the rat or during connection to the perfusion pump. To further prevent destruction by the rat, the needle-buttressed ends were brought out no more than a few millimeters from the cutaneous incision in the back of the neck. When in use, the catheters were connected to PE-90 tubing from syringe pumps via hollow shafts made from no. 20 hypodermic needles. Because the rats were restrained during sham feeding, they could not disrupt the perfusion system.

In the naturally feeding, tethered rat model, perfusion catheters were similarly inserted into proximal duodenum and midgut, but the proximal ends of the catheters exited the neck through a flanged tube of hard plastic. The flange of this tube was fenestrated to allow its suturing to an overlying strip of arterial graft Dacron mesh. Next, the Dacron wings of the assembly were sutured at multiple sites to the posterior neck muscles below the skin, and the skin was closed over the assembly. As with the sham-fed rats, the catheters were connected during use to PE-90 tubing from syringe pumps, but in this case, the pump tubings were protected from disruption by the rat with an overtube of wound wire that twisted firmly onto the shaft of the hard plastic exit tube. On the proximal end, the assembly of wound wire overtube plus inner pump tubes was suspended from a swivel that in effect allowed the rat to wander naturally about its cage without opportunity for disrupting flow while being perfused during the 3-h feeding period. Unlike the sham-fed rats, the naturally feeding rats had no gastric fistulas.

All animals were in good health while being studied, but, not infrequently, the rats had to be reoperated on to splice subcutaneously a new section of perfusion catheter onto the original catheter that the animal had pulled partially out and severed.

Feeding Routines and Experimental Protocols

Animals were housed in individual cages in a room with a natural light cycle. Sham-fed rats were trained to eat their day’s ration from 1200–1700 and were then deprived of food but not water from 1700–0900 the next day. These animals were studied sometime in the interval from 0900–1200. Naturally feeding, tethered rats were trained to eat daily from 0900–1200 or from 1300–1600 and were studied in their assigned feeding period. For the remaining 21 h, the tethered rats were deprived of food but not water.

We have used the term “naturally feeding rats” throughout this and companion papers (27, 28) to distinguish this model from sham-feeding animals. In contrast to the sham-feeding animals, the naturally feeding rats were unrestrained in a standard cage environment during feeding, ate usual rat chow during the tests, and did not have ingested nutrients diverted from the gastrointestinal tract. Nevertheless, these animals were fasted 21 h each day before the feeding period, and the feeding period was constrained to 3 h, so their feeding was not truly “natural.”

To prevent evulsions of gastric cannulas and to facilitate collection of gastric effluent, sham-fed rats were studied in plastic restraining cages. The fasted animals were brought to the laboratory and, at the desired time from 0900 to 1200, were placed into the restraining cage. The gastric cannula was then opened by unscrewing its cap, and the stomach was washed with saline until the return was clear. The perfusion catheters were then connected to the pump tubing. After 20 min, the rat was given free access to a nipple valve from a bottle that contained 11% (wt/wt) of aqueous Polycose (Ross Feeds, St. Louis, MO) was placed and anchored inside the laboratory each weekday, removed from their permanent cages, and placed into cubical wire cages with drop-through catch basins at the bottom. Their perfusion catheters were connected to pump tubings that entered the cage through the fenestrated wire top. A tethered assembly allowed the rats to roam naturally about their cage without opportunity for disrupting flow during the 3-h feeding period. At the start of the feeding period, a feeding dish with chow during the tests, and did not have ingested nutrients diverted from the gastrointestinal tract. Nevertheless, these animals were fasted 21 h each day before the feeding period, and the feeding period was constrained to 3 h, so their feeding was not truly “natural.”

To prevent evulsions of gastric cannulas and to facilitate collection of gastric effluent, sham-fed rats were studied in plastic restraining cages. The fasted animals were brought to the laboratory and, at the desired time from 0900 to 1200, were placed into the restraining cage. The gastric cannula was then opened by unscrewing its cap, and the stomach was washed with saline until the return was clear. The perfusion catheters were then connected to the pump tubing. After 20 min, the rat was given free access to a nipple valve from a bottle that contained 11% (wt/wt) of aqueous Polycose (Ross Laboratories, Columbus, OH; nutritional value = 3.83 kcal/g dry powder), and the syringe pumps were immediately started. The period of sham feeding was 90 min, after which we measured 1) grams of Polycose consumed (outcome) and 2) grams of fluid collected beneath the draining gastric cannula. If the effluent from the stomach was <85% of the weight of the Polycose drunk, the experiment was discarded and repeated on another day. Animals were trained until they consumed daily a large (50 ml), constant volume of Polycose during sham-feeding experiments that continued for 10–14 days of training, and then they were started on sham feeding experiments. Routinely, we studied sham-fed rats four mornings each week (Tuesday–Friday), giving them their day’s ration of rat chow in the afternoons. During weekends, daytime feedings continued; the animals were fasted on Sunday evening, and each Monday morning, the rats were reintroduced to a training period of sham feeding. These sham-fed animals had useful experimental lives of ~3 wk after which they either disrupted their cannulas beyond repair or grew weary of the sham feeding routine so as to no longer accept the confinement in restraint cages.

Fasted, naturally feeding tethered rats were brought to the laboratory each weekday, removed from their permanent cages, and placed into cubical wire cages with drop-through catch basins at the bottom. Their perfusion catheters were connected to pump tubings that entered the cage through the fenestrated wire top. A tethered assembly allowed the rats to roam naturally about their feeding cages during the 3 h of feeding. At the start of the feeding period, a feeding dish with a preweighed amount of powdered rat chow (Richmond Standard, 5001 Laboratory Rodent Diet, 3.4 kcal/g; PMI Feeds, St. Louis, MO) was placed and anchored inside the cage so that the rat was allowed unrestricted access to a large excess of rat chow. A water bottle was inserted at one end of
the cage to give the animal free access to drinking water. At
the end of the 3 h, the rat was disconnected from the pump
and returned to its permanent cage. Any dry food that
spilled onto the catch basin below the cage was returned to
the feeding dish, and the weight of chow consumed was
determined by difference from the initial weight. If dropped
food was soaked in urine or diarrhea, its weight was deter-
mined after drying under an infrared lamp and subtracted
from the difference between initial and final bowl weights. To
eliminate progressive weight loss, rats were perfused only 2
or 3 days a week, with 1–3 days of undisturbed eating
between days of perfusion. Control intakes were measured on
these days of nonperfusion. Useful experimental life of this
preparation ranged from 1 to 14 mo, until the perfusion
catheters were disrupted beyond repair.

Perfusions

Except for solutions of carbohydrates, perfusates were
made isosmolar by adding NaCl as needed to achieve 300
mosmol/kgH2O. For solutions of carbohydrates, osmolalities
were set at 400 for duodenal instillates and 800 for midgut
infusions, because we found in preliminary experiments that
solutions of NaCl alone markedly inhibited intakes at osmo-
alities above, but not below, 500 mosmol/kgH2O in duodenum
solutions of NaCl alone markedly inhibited intakes at osmo-
alities above, but not below, 500 mosmol/kgH2O in duodenum
and above, but not below, 800 mosmol/kgH2O in ileum. Oleate
plus polycose was emulsified with 10 mM taurocholate at
pH 7 in a 2:1 molar ratio (oleate:taurocholate). Control solutions
were NaCl plus 10 mM taurocholate. All solutions at 21°C
were instilled at pH 7, except those for decanoate (pH 7.5) and
dodecanoate (C-12, pH 8.1–8.5). With each model, perfusion
began at the start and continued for the duration of the
ingestive period.

Doses were scheduled in Latin square designs among
animals in each group. In most instances, when dose re-
sponses were to be compared for duodenal vs. midgut
infusions, one-half the animals were perfused first at duodenum
while the other one-half began with instillations into midgut.
After the first dose response was completed, the sites were
reversed for the next dose response. For sham-fed animals,
each dose was administered one time at each site. For
naturally fed animals (which had much longer experimental
lives), dose-response curves at each site were determined two
times in most rats, and the responses were averaged to give
one value for each animal at each dose.

Acarbose, a competitive inhibitor of mucosal α-1,4-glucosi-
dase (23), was perfused in some experiments along with
maltose or Polycose. Tablets of Precose (acarbose; Bayer)
were crushed and dissolved in the perfusates at 25 mg/ml;
inactive ingredients (talc, magnesium stearate) were re-
moved by centrifugation before perfusion.

Analyses of Results

Two-way ANOVAs were used to determine whether there
was a significant treatment effect among perfusions with
control and all doses. If so, dose-responsive effects were
examined by linear regression of values in each animal, with
0 dose = control and successive doses (given in geometric
progression) arrayed arithmetically as doses 1, 2, 3, and 4
(when applicable). Significance (difference from zero by t-test)
of slopes (g ingested/U dose) was then computed from the
mean of values from all animals. For global comparisons
of one dose response to another, we used paired t-tests to
compare slopes (from proximal vs. distal perfusions or be-
tween two perfusates) within the same animals and also the
sum of responses to all doses (10).

For both sham-fed and naturally feeding rats, percent
inhibitions were calculated as 100 – (100 × the response
during nutrient dose ÷ by the response during control). In the
naturally feeding, tethered rats, “reduction ratios” were
calculated as 100
$\frac{2}{3}$
(10).

RESULTS

Sham-Fed Rats

Perfused fatty acids. In the first set of experiments (Fig. 1A), 12 rats were sham fed while saline (control)
or 20–80 mM oleate-monolein was perfused at 12 ml/h.
Doses were expressed as millimoles per hour (concentra-
tions × hourly volumes). At either site, there was a
significant (P < 0.001) dose response, but there was no
significant difference (P > 0.1) between dose responses at
duodenum vs. midgut.

To test whether the response varied with total load
rather than concentration of fatty acid, loads were
varied at fixed concentrations in nine animals by
infusing 80 mM oleate-monolein at 3, 6, and 12 ml/h
compared with a saline control at 12 ml/h. Seven of
the nine rats had been used in the preceding experiment.
There were again significant dose responses (P < 0.005)
at each site, and, again, there was no significant
difference between the slopes of the dose responses
from duodenal vs. midintestinal infusions (Fig. 1B).
In general, the shapes of the dose responses were similar
to those in the preceding experiment. In fact, there
were no significant differences (unpaired t-tests) be-
tween the slopes of the dose responses when the same
ranges of loads of oleate were infused at constant flow
vs. constant concentration at either site.

Next C-12 was infused into duodenum or midgut at
12 ml/h in concentrations of 20, 40, and 80 mM. On
duodenal infusion (Fig. 1C) in 9 animals, the C-12
evoked a significant dose response (P < 0.005) quite
similar to that of oleate (Fig. 1A). However, on infusion
of C-12 into midgut, there was not a statistically
significant treatment effect because one of the animals
ingested nothing during the control perfusion with
saline. If this outlier (>2.5 SD from the mean) was
eliminated from the analyses, C-12 gave significant
dose responses (P < 0.005) at each site, and the
responses to duodenal infusions did not differ from
those during midgut infusions.

The effect of fatty acid chain length on potency for
inhibition of sham feeding was examined by separately
infusing sodium salts of octanoic (C-8), decanoic (C-10),
and dodecanoic acid at 12 ml/h into the duodenum
(Table 1). Even though C-12 inhibited sham feeding (com-
pared with perfusions with saline controls), nei-
ther isocaloric amounts of C-8 nor equimolar loads of
C-10 reduced intakes below control.

Inhibitions by sugars. To compare inhibition from
duodenum vs. midgut, we infused dimeric maltose in
nine rats (Fig. 2A). Maltose is hydrolyzed at the mucosa to two molecules of glucose faster than glucose is absorbed. Indeed, we found that isocaloric loads of duodenal maltose or glucose inhibited intakes about equally. We used maltose rather than glucose in most experiments so that we could limit both osmolality and flow rates, yet infuse significant calories. At both proximal and distal sites, the 100–400 mM maltose inhibited sham feeding in a dose-related fashion, and there was no significant difference between the slopes of the proximal and distal dose responses.

To determine whether inhibition was chemically specific to glucose, we studied other monomeric sugars. D-Xylose significantly inhibited sham feeding in a dose-dependent fashion ($P < 0.005$) when infused into duodenum (Fig. 2B) but was ineffective on infusion into midgut. Although the slope of the duodenal dose response ($-10.3 \pm 2.2$) was numerically much more negative than that from the midgut infusions ($-3.9 \pm 2.8$), the difference was not statistically significant ($P \leq 0.1$). Neither duodenal fructose, over the range of 1.2–4.8 mmol/h (Fig. 2C), nor midintestinal fructose (1.2–9.6 mmol/h) significantly inhibited intakes (i.e., no treatment effect, no dose response). Nonabsorbable D-mannose (a stereoisomer of glucose) and each of the two glucose analogs (3-O-methylglucose and $\alpha$-methylglucose) were instilled into the midintestine at 12 ml/h in concentrations ranging from 200 to 800 mM (Fig. 2, D and E). Over these ranges of doses, both the 3-O-methylglucose and the $\alpha$-methylglucose were potent, but the mannose did not significantly inhibit sham feeding.

Amino acids. Previously, intestinal L-phenylalanine (41) had been identified as a potent intestinal inhibitor of sham feeding in rats. To confirm this finding, we perfused 62.5–250 mM phenylalanine at 12 ml/h into the duodenum and midintestine in eight animals. The L-phenylalanine was not clearly potent (Fig. 3A), that is, there was no significant effect ($P > 0.10$) for the duodenal infusions but a significant treatment effect ($P < 0.025$) and dose response for the midgut infusions.

Four other L-amino acids were examined for potency by infusing them vs. control infusions of saline into the duodenum of eight rats (Table 2). For arginine and alanine, 3 mmol/h were instilled at 250 mM concentrations, but leucine was so insoluble that this load could be achieved only by infusing 125 mM solutions at 24 ml/h. Because tryptophan was even more insoluble, it was instilled at a load of 1.5 mmol/h (62.5 mM $\times 24$ ml/h). In each case, the control infusions of 0.15 M NaCl were given at the same rate of flow as the amino acid solution. Only the tryptophan inhibited significantly.

### Table 1. Fatty acids on sham feeding in rats

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>No. of Rats</th>
<th>Amount Ingested, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (0.15 M)</td>
<td>14</td>
<td>$62 \pm 8$</td>
</tr>
<tr>
<td>Dodecanoate (80 mM)</td>
<td>14</td>
<td>$28 \pm 7^*$</td>
</tr>
<tr>
<td>NaCl (0.15 M)</td>
<td>8</td>
<td>$55 \pm 9$</td>
</tr>
<tr>
<td>Decanoate (80 mM)</td>
<td>8</td>
<td>$72 \pm 11$</td>
</tr>
<tr>
<td>NaCl (0.15 M)</td>
<td>12</td>
<td>$67 \pm 8$</td>
</tr>
<tr>
<td>Octanoate (120 mM)</td>
<td>12</td>
<td>$75 \pm 10$</td>
</tr>
</tbody>
</table>

Responses are means $\pm$ SE of ml ingested over 90 min of sham feeding while fatty acid or saline was infused at 12 ml/h into duodenum. Animals that did not respond to decanoate or octanoate were part of the group of 14 rats that did inhibit when tested during duodenal perfusion of dodecanoate vs. saline. *Only dodecanoate inhibited significantly more than saline controls ($P < 0.0005$, paired t-test).
The 250 mM l-phenylalanine not only was of questionable potency but also was a supersaturated solution, which, on cooling, tended to crystallize and plug the PE-90 perfusion catheters. To develop a more potent amino acid stimulus, we combined 180 mM l-phenylalanine with 60 mM l-tryptophan. The mixture was perfused into duodenum or midgut of 10 rats at 3, 6, and 12 ml/h, and its effects were compared with control solutions of 0.15 M NaCl at 12 ml/h (Fig. 3B). Again, there was no significant treatment effect on duodenal infusion, but the infusion at midgut did show such an effect (P, 0.025).

Naturally Feeding Rats

Responses to nutrients and nutrient analogs. In preliminary studies in six animals, daily intakes of rat chow did not significantly differ whether the animals were repeatedly observed during no perfusion (14.3 ± 0.5 g/day) or during perfusion at 12 ml/h into duodenum with 0.15 M NaCl at 12 ml/h (Fig. 3B). Therefore, we elected not to perfuse the animals during control days to reduce manipulation of the perfusion catheters and thus to preserve them as long as possible. Intakes of nonperfused animals were fairly constant whether or not the animals had been perfused on the preceding day, and control intakes in each animal remained constant over the entire period of observation. Therefore, control intakes were averaged from intakes on every control day of no perfusion for each animal, and each animal then contributed one value to the group means and SE for all animals. Calculations of reduction ratios or percent inhibition, however, were made for individual animals on the basis of the animal’s own control intake.

All four nutrients significantly inhibited natural feeding in a dose-related fashion (P < 0.005 for all except midgut maltose for which P < 0.025), whether infused into duodenum or midintestine (see Fig. 4). For maltose (P < 0.025) and even more clearly for oleate-monolein (P < 0.0005), the slopes of the dose response to duodenal perfusions were significantly more negative than slopes to perfusion at midgut (paired t-tests). In the case of oleate-monolein, this difference was due almost entirely to a greater inhibition on infusion of the highest dose at duodenum compared with midgut. For maltose, the difference in slopes between proximal and distal infusions was the result of a combination of slightly less inhibition at lower doses and somewhat more inhibition at the highest dose during instillations at duodenum compared with midgut (Fig. 4).

To confirm and extend the observations from the sham-feeding studies, we also examined inhibition of natural feeding by midintestinal, 800 mM, 3-O-methylglucose, α-methylglucose (9.6 mmol/h), or 400 mM...
D-xylose (4.8 mmol/h) perfused into duodenum or into midgut. On infusion into mid small intestine, the 3-O-methylglucose and α-methylglucose significantly (P, 0.005, paired t-test) inhibited feeding; but the D-xylose did not. However, the 4.6 mmol/h of duodenal D-xylose significantly (P, 0.005) reduced food intake (Table 3).

Elsewhere (28), we have observed that varied loads of duodenal glucose about as effectively suppressed food intakes as isocaloric doses of dimeric maltose. We wondered how efficacy of even longer glucose polymers would compare with isocaloric maltose. Therefore, we compared isocaloric loads of Polycose (a mixture of glucose polymers 2–25 glucose units in length, with an average length of 5 glucose units) with maltose on duodenal infusion (in 12 animals) and again on midintestinal infusion (in 10 rats). On duodenal infusion, the Polycose exhibited a slope for its dose response that insignificantly differed from that of maltose (Fig. 5). Nevertheless, the Polycose was overall somewhat more effective, as the sum of responses was lower (P < 0.025). On infusion into midgut, the Polycose was more clearly effective than maltose, as the slope of its dose response was significantly (P < 0.05) more negative and the sum of responses significantly (P < 0.01) lower than those of maltose.

These differences raised the possibility that there were sensors with high specificities for longer glucose polymers. To check for this possibility, we examined the suppression of food intakes after high duodenal loads (7 kcal/h) of maltose and of Polycose in the presence vs. the absence of effective doses of the glucosidase inhibitor acarbose. The acarbose greatly reduced the suppression for either carbohydrate but was without effect on suppression from isocaloric glucose (Table 4). Participation of regions of bowel. The above observations suggested that proximal and distal halves of small intestine were about equally responsive to infused nutrients. We undertook additional experiments to examine the likely roles of participating segments of bowel.

EFFECT OF RESECTION OF ILEUM ON DOSE RESPONSE. We resected the distal one-half of small bowel, surgically joining the transected jejunum to the terminal ileum just proximal to the ileocecal valve. The five surviving animals with ileal resections had a duodenal infusion catheter, just as the unresected rats. Despite the ileal resection, there were still dose-responsive inhibitions by each of the four nutrients during duodenal instillations (significant treatment effects P < 0.01; significant dose response, P < 0.01). Moreover, the slopes of the dose-response curves in these five resected animals did not differ significantly (unpaired t-test) from the corresponding slopes of the duodenal dose responses in the 9–10 unresected rats, but there was a tendency for the dose-response curves in the resected animals to parallel but lie above the corresponding curves in the unresected rats (Fig. 6). This difference was significant only for oleate-monolein, that is, the sum of percent inhibition for the 0.48 and 0.96 mmol/h doses on duodenal instillation in the intact animals was significantly higher (P < 0.025, unpaired t-test) than that from duodenal instillations in the resected animals.

INFUSIONS TO ASSESS WHETHER THE COLON CONTRIBUTED TO DOSE RESPONSE. In an additional six tethered, naturally feeding animals, we implanted an infusion catheter into the cecum, so that we could determine the effects of perfusing the colon on food intakes (Table 5).
The animals were perfused at 6 ml/h, a rate that seldom induced diarrhea over the 3 h of observation during perfusion with 0.15 M NaCl. With each nutrient, animals were tested two times with saline perfusions (controls) on days before and after the day of perfusion with nutrient. The order of nutrients was randomized among the six animals. All four nutrients significantly (P < 0.01) inhibited food intake on colonic perfusion. Most, but not all, of the animals developed diarrhea of varying severity and time of onset during the 3 h of perfusion with the fatty acids or maltose, but not with the phenylalanine plus tryptophan. We graded the diarrhea as 0 (absent), 1 (mild; that is mostly solid stool with some moistening), 3 (heavy; that is voluminous watery stools), and 2 (moderate, between mild and heavy). There was no correlation between the percent of inhibition in individual animals and the diarrhea scores among any of the individual nutrients or across all of the tests.

### Time courses and duration of effects.

Our naturally feeding perfused rat model was new, so there was no information hitherto on the time courses of inhibition of food intakes during intestinal perfusions with nutrients. In our first experiments to examine time courses, we perfused eight rats at midintestine with α-methylglucose, glucose, dodecanoate, or phenylalanine-tryptophan. We graded the diarrhea as 0 (absent), 1 (mild; that is mostly solid stool with some moistening), 3 (heavy; that is voluminous watery stools), and 2 (moderate, between mild and heavy). There was no correlation between the percent of inhibition in individual animals and the diarrhea scores among any of the individual nutrients or across all of the tests.

### Table 3. Percent inhibition of gut perfusates

<table>
<thead>
<tr>
<th>Dose, mmol/h</th>
<th>Duodenum</th>
<th>Midgut</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham fed</td>
<td>Naturally fed</td>
</tr>
<tr>
<td></td>
<td>Sham fed</td>
<td>Naturally fed</td>
</tr>
<tr>
<td>Maltose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>19.8</td>
<td>0.0</td>
</tr>
<tr>
<td>2.4</td>
<td>23.6</td>
<td>0.0</td>
</tr>
<tr>
<td>4.8</td>
<td>58.6</td>
<td>52.9</td>
</tr>
<tr>
<td>Oleate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>19.9</td>
<td>5.2</td>
</tr>
<tr>
<td>0.48</td>
<td>87.1</td>
<td>37.2</td>
</tr>
<tr>
<td>0.96</td>
<td>89.4</td>
<td>80.1</td>
</tr>
<tr>
<td>Dodecanoate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.48</td>
<td>42.5</td>
<td>11.3</td>
</tr>
<tr>
<td>0.96</td>
<td>78.2</td>
<td>63.4</td>
</tr>
<tr>
<td>Phenylalanine+tryptophan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.72</td>
<td>0.0</td>
<td>1.3</td>
</tr>
<tr>
<td>1.44</td>
<td>5.1</td>
<td>34.8</td>
</tr>
<tr>
<td>2.88</td>
<td>34.7</td>
<td>40.4</td>
</tr>
<tr>
<td>Other sugars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-xylose (4.8 mmol/h)</td>
<td>39.6</td>
<td>38.2</td>
</tr>
<tr>
<td>3-O-methylglucose (9.6 mmol/h)</td>
<td>86.9</td>
<td>59.7</td>
</tr>
<tr>
<td>α-methylglucose (9.6 mmol/h)</td>
<td>48.9</td>
<td>89.0</td>
</tr>
</tbody>
</table>

*Estimates made from 100 – [100(ingested during perfusion of nutrient/ingested during perfusion of saline)]. If the same or more was ingested during nutrient compared with saline perfusion, %inhibition was designated as 0.0.

### Fig. 4. Effect of infusing different doses of 4 nutrient solutions into duodenum or midgut on daily intakes of food (means ± SE) in naturally feeding rats. N, no. of animals studied. Control intakes (at dose = 0) are grams of rat chow consumed by the animals on days of no perfusion.
Perfusions were stopped after 1.5 h, and we measured intakes separately for the initial and final 1.5 h of the 3-h feeding period. Intakes were compared during and after each 1.5-h perfusion with control intakes during no perfusion. The doses of perfusates had been predetermined to give about the same suppression intakes (to 8–9 g of food consumed) during a full 3 h of perfusion. The point of this first experiment was to determine whether the glucose, C-12, or amino acids had a more sustained effect after perfusions were stopped than the nonmetabolized α-methylglucose. The effects of neither the glucose nor the dodecanoate differed from those of nonmetabolized methylglucose, either during the initial 1.5 h of perfusion or afterward (Table 6), but the phenylalanine-tryptophan suppressed intakes less during the initial 1.5 h of infusion and more in the later period after the infusion was stopped. This pattern suggested a cumulating effect of the infused amino acids compared with the other perfusates. However, intakes from 1.5 to 3.0 h in the control experiments were much lower than from 0 to 1.5 h. Therefore, it was not clear whether low spontaneous intakes from 1.5 to 3 h masked some persisting effects of the fatty acids or the glucose.

Experiments were repeated to see whether the perfusates would have persisting effects on the high rates of spontaneous intake that characterized the early period of feeding, that is, under conditions in which the animals would be more sensitive to persisting effects. We gave high doses of maltose, C-12, C-18, or phenylalanine-tryptophan into the duodenum for 1.5 h either immediately before the beginning of the 3-h feeding period or, in a second iteration, for 1.5 h, ending 1 h before the start of feeding (Fig. 7). When perfusions were stopped just before eating began, maltose had no persisting effect. By contrast, the amino acids, dodecanoate, or oleate continued to significantly (P < 0.05, 2-way ANOVA) suppress intakes over the first hour after stopping the perfusions (Fig. 7A). The initial suppression was less for the phenylalanine plus tryptophan than for the fatty acids. In the second and third hours after stopping the perfusions, eating after the fatty acids accelerated, but eating was still suppressed after the amino acids, so that the gaps between control and perfusion experiments narrowed in the second and third hours after fatty acid perfusions but widened after perfusions with amino acids (Fig. 7A).

When 1.5-h perfusions were stopped 1 h before eating began, there was still a significant suppression of intakes (compared with control) in the first hour of eating after oleate, that is, over hours 1–2 after stopping oleate. First-hour reductions (compared with control) after decanoate or phenylalanine plus tryptophan were no longer significant. By the end of the second hour of eating (3 h after stopping the perfusates), there were no significant differences among intakes after control experiments or any of the perfusates (Fig. 7B).

Reduction ratios. The naturally feeding model uniquely allowed calculation of the efficiency with which the various nutrients inhibited intakes of calories from the rat chow during the 3-h feeding as reduction ratios (Table 7). At the lowest doses for all four nutrient infusions, inhibition of food intake was “inefficient,” that is, the reduction ratios were <1.0. For the dodecanoate, reduction ratios rose linearly and steeply over the range of the three geometrically increased doses. For oleate-monolein, there was a steep
rise between the lowest and middle doses, with the ratio maximum at the middle dose. But for maltose the reduction ratio rose between the middle and highest dose, never reaching the high values of the other nutrients.

**DISCUSSION**

Sham-Fed vs. Naturally Fed Models

There was a good correspondence between the results in the sham-fed animals and the observations in the naturally feeding tethered rats, the same nutrients or nutrient analogs inhibited in both models with similar dose-responsiveness, and results of comparisons of proximal to distal intestinal responsiveness were similar in each model (Table 3). Nevertheless, sham-fed rats were more sensitive than naturally feeding animals to lower does of nutrients, whereas naturally feeding animals were more responsive to duodenal phenylalanine plus tryptophan. Whether or not

Table 5. Colonic perfusions on food intakes

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Dose, mmol/h</th>
<th>Control</th>
<th>Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecanoate</td>
<td>0.48</td>
<td>13.2 ± 0.9</td>
<td>8.0 ± 0.4</td>
</tr>
<tr>
<td>Oleate-monoinein</td>
<td>0.48</td>
<td>13.8 ± 0.6</td>
<td>10.4 ± 1.3</td>
</tr>
<tr>
<td>Maltose</td>
<td>2.4</td>
<td>13.1 ± 1.2</td>
<td>10.3 ± 1.2</td>
</tr>
<tr>
<td>Phenylalanine+ tryptophan</td>
<td>1.4</td>
<td>15.1 ± 0.6</td>
<td>11.1 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE for intakes (g) of chow in 6 rats. Two-way ANOVAs with contrasts indicated that intakes during colonic perfusions with each of the four nutrients were significantly less (P < 0.01) than during control perfusions with 0.15 M NaCl.

Table 6. Suppression and persistence of suppression of food intakes

<table>
<thead>
<tr>
<th>Mode (Time):</th>
<th>Midgut Perfusion (0–1.5 h)</th>
<th>No Perfusion (1.5–3 h)</th>
<th>Total Intake (0–3 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.8 ± 0.8</td>
<td>5.2 ± 0.5</td>
<td>17.1 ± 0.8</td>
</tr>
<tr>
<td>Glucose (0.8 M)</td>
<td>4.2 ± 0.6*</td>
<td>7.3 ± 1.1</td>
<td>11.5 ± 1.4*</td>
</tr>
<tr>
<td>α-Methylglucose (0.8 M)</td>
<td>4.7 ± 0.4*</td>
<td>7.6 ± 0.8</td>
<td>12.3 ± 1.1*</td>
</tr>
<tr>
<td>Dodecanoate (0.06 M)</td>
<td>4.2 ± 0.9*</td>
<td>7.8 ± 1.3</td>
<td>12.0 ± 1.7*</td>
</tr>
<tr>
<td>Phenylalanine-tryptophan (0.24 M)</td>
<td>5.9 ± 0.7*</td>
<td>2.7 ± 0.9†</td>
<td>8.6 ± 0.9*</td>
</tr>
</tbody>
</table>

Data are means ± SE of grams of rat chow eaten from 8 rats. All perfusates were delivered into midgut at 12 ml/h from 0 to 1.5 h of the 3 h experiments but then were stopped while food intakes were again measured from 1.5 to 3 h. In contemporaneous, control experiments on alternate days, the animals were not perfused, but their intakes were measured during the first and second halves of the 3-h daily feeding periods. Control data are the averages from 3 days of such observations. *Significantly less (P < 0.01, 2-way ANOVA with 4 contrasts) than corresponding control intake. †Significantly less (P < 0.05) than corresponding control intake and less (P < 0.01) than average of corresponding intakes after stopping each of the other three perfusates (2-way ANOVA with 2 contrasts).
not results with phenylalanine plus tryptophan were included in the analysis, there was a significant (P < 0.0001) correlation between dose-related suppression of intakes (as a percent of control) in sham- vs. naturally fed animals such that perfused nutrients were ~70% as effective at suppressing intakes in naturally fed animals when compared with sham-fed rats.

With both models, rats were tested after >15 h of fasting, the usual situation with sham-feeding rats. Just as in sham-fed animals (31), the prolonged, pretest fasting produced higher initial rates of control intakes in the tethered rats (Fig. 7 and Table 6) than commonly observed in short-term experiments with continuously feeding animals (30). In control tests, 3-h food intakes were high despite the high volumes (27) of food and secretions distending the stomach of the tethered rats. This observation suggests that the potent effect of much smaller intragastric volumes on inhibiting short-term intakes in continuously feeding rats (2) was overridden by hunger after prolonged fasting.

There were two important physiological differences between the models. 1) There was no significant amount of food in the gastrointestinal tract of sham-fed rats (because the stomachs were continuously drained), whereas food was obviously present in the stomach and to an unknown extent in the intestines of the tethered animals. 2) Tethered rats were perfused with intestinal nutrients twice as long as sham-fed animals. That our intestinal perfusions with nutrients in the tethered rats so effectively suppressed even initial intakes in this setting (Table 6) suggests that reductions of intakes.

### Table 7. Reduction ratios

<table>
<thead>
<tr>
<th>Dose</th>
<th>mmol/h</th>
<th>kcal/h</th>
<th>Duodenum</th>
<th>Midgut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>1.7</td>
<td>-0.88±0.35</td>
<td>0.02±0.59</td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>3.5</td>
<td>-0.38±0.24</td>
<td>-0.04±0.23</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>6.9</td>
<td>1.41±0.14†</td>
<td>1.03±0.14†</td>
<td></td>
</tr>
<tr>
<td>Polycose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.24)</td>
<td>1.7</td>
<td>0.12±0.44</td>
<td>0.81±0.79</td>
<td></td>
</tr>
<tr>
<td>(0.48)</td>
<td>3.5</td>
<td>0.78±0.50</td>
<td>1.61±0.37†</td>
<td></td>
</tr>
<tr>
<td>(0.96)</td>
<td>6.9</td>
<td>1.57±0.14†</td>
<td>1.33±0.08†</td>
<td></td>
</tr>
<tr>
<td>Oleate‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>3.0</td>
<td>0.95±0.43</td>
<td>0.46±0.76</td>
<td></td>
</tr>
<tr>
<td>0.48</td>
<td>5.9</td>
<td>3.37±0.65†</td>
<td>2.94±0.47†</td>
<td></td>
</tr>
<tr>
<td>0.96</td>
<td>11.9</td>
<td>3.58±0.14†</td>
<td>2.74±0.24†</td>
<td></td>
</tr>
<tr>
<td>Dodecanoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>1.3</td>
<td>-0.83±1.03</td>
<td>0.06±2.11</td>
<td></td>
</tr>
<tr>
<td>0.48</td>
<td>2.6</td>
<td>2.22±1.44</td>
<td>4.49±1.23†</td>
<td></td>
</tr>
<tr>
<td>0.96</td>
<td>5.2</td>
<td>6.96±0.70†</td>
<td>6.33±0.53†</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + Tryptophan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.72</td>
<td>1.7</td>
<td>0.35±2.12</td>
<td>-4.23±2.14</td>
<td></td>
</tr>
<tr>
<td>1.44</td>
<td>3.4</td>
<td>5.63±1.60*</td>
<td>3.58±0.60†</td>
<td></td>
</tr>
<tr>
<td>2.88</td>
<td>6.8</td>
<td>3.22±0.59†</td>
<td>3.14±0.79*</td>
<td></td>
</tr>
<tr>
<td>5.76</td>
<td>13.6</td>
<td>3.07±0.28†</td>
<td>2.65±0.18†</td>
<td></td>
</tr>
</tbody>
</table>

Data are means = SE for 6–13 animals. Reduction ratios are control intake (kcal) – intake during nutrient perfusion (kcal) divided by kcal infused during nutrient perfusions. Negative values indicate that intakes were higher during nutrient infusion than during control studies; a zero value indicates no reduction of intake during nutrient infusion, and positive values indicate that food intakes were less during nutrient infusions than in control tests. Approximate molar doses for Polycose, a mixture of polysaccharides of inexact molar composition, are indicated in parentheses.

* Different from 0, P < 0.05. † Different from 0, P < 0.01. ‡ Molar dose of oleate is determined from content of oleic acid, but kcal dose is based on content of both oleic acid and monolein.
takes signalled by intestinal nutrients can be more potent than cues from gastric distension. Furthermore, that dose-responsive inhibitions of nutrient intakes were similar in sham-fed (stomach empty) and naturally fed (stomachs distended) animals indicates that nutrient-driven feedback from intestine can independently inhibit food intake, without the necessity of simultaneous gastric distension or nutrient-driven inhibition of gastric emptying. The tethered rats were perfused twice as long as the sham-fed animals, so it is possible that a more prolonged accumulation of infused nutrients in gut lumen, gut wall, and/or systemic compartments could have differently affected intakes in the naturally feeding rats. That there was such a strong correlation between outcomes in the two models suggests, however, that this potential difference was not important. Indeed, except with phenylalanine plus tryptophan, we could adduce only weak and fleeting effects of accumulation in our temporal analyses (Fig. 7 and Table 6).

Chemical Specificities

Carbohydrates. Our experiments with a series of sugars confirm and extend previous observations. Previously, D-xylose had been recognized to potently inhibit natural or sham feeding in rats (40), monkeys (29), and humans (33), whereas fructose was not nearly as satiating as glucose in rats (30) and monkeys (29). We found that duodenal D-xylose was at least as potent on inhibiting sham feeding (Fig. 2) or natural feeding (Table 3) as isocaloric maltose. On the other hand, fructose did not significantly inhibit sham feeding (Fig. 2). D-XYlose is known to be transported by the mucosal glucose transporter, whereas fructose is not. In other experiments in naturally feeding rats (28), we found that galactose inhibited intakes nearly the same as glucose. Galactose is also absorbed via the glucose transporter. To test further the idea that occupation of the glucose transporter may evoke a satiety signal, we studied 3-O-methylglucose [as did Booth (2)] and α-methylglucose. Both substituted glucoses have high affinities for and are transported by the mucosal glucose transporter (16), and both inhibited sham feeding (Fig. 2) and natural feeding (Table 3) with about the same potency as isocaloric maltose. Finally, the isomer of glucose, D-mannose, did not inhibit sham feeding (Fig. 2), nor is this sugar transported by the glucose transporter. Thus all observations indicated that food intake is suppressed by monomeric sugars only with affinity for the glucose transporter.

Although duodenal D-xylose was as potent or more potent than isocaloric maltose, midintestinal D-xylose was impotent (Fig. 2 and Table 3), an anomaly among sugars, as glucose or galactose (not shown) and maltose were equipotent on infusion at either site. D-xylose is transported by the glucose transporter, but it has a low affinity (18), so that its maximal rate of intestinal absorption is slow, <25% of that for glucose (8). Because response appears to be dominated by length of intestinal contact with sugar (28), it is likely that D-xylose acted by occupying the glucose transporter, but its affinity was so low that the satiety response it elicited was lower per centimeter of bowel than that to maltose. On the other hand, its low satiety per centimeter was counterbalanced by its slow absorption and thus greater length of contact on duodenal instillation.

D-xylose is only 25% metabolized (8), 3-O-methylglucose is only 2% metabolized (7), and α-methylglucose likewise is not metabolized (21). Conversely, fructose has little effect on food intakes, although it is fully metabolized (Fig. 2 and Ref. 29). This catalogue of potencies among sugars indicates that suppression is signalled by specific sensors in small intestine, not through their postabsorptive metabolism. Thus intravenous glucose, although fully metabolizable, is not nearly as satiating as isocaloric, intraduodenal glucose (34, 36).

Both the disaccharide, maltose, and the polysaccharide, Polycose (with an average of 5 glucose units), were potent on duodenal or midintestinal infusion (Fig. 5). Both carbohydrates evidently acted after hydrolysis at the mucosa to glucose, because acarbose, a competitive inhibitor of mucosal glucosidases, significantly and markedly reduced suppression of food intake during infusion of either carbohydrate (Table 4), just as Maggio and Vasselli (23) had previously observed that acarbose nearly abolished reductions of food intakes in rats by premeals of starch.

The nearly similar efficacies of duodenal glucose (28), dimeric maltose, and polymeric Polycose on suppressing food intakes in naturally feeding rats resembled the equipotencies of isocaloric premeals of starch or glucose on inhibiting food intakes in monkeys (24). Both observations on satiety parallel similar findings of equal inhibition of gastric emptying by isocaloric meals of glucose or starch (16). Several observations indicate (28) that each of these responses is determined by length of intestinal contact with glucose products. Progressive hydrolysis of starch by luminal amylases and mucosal saccharidases is so rapid that length of intestinal contact with released glucose is determined by maximal glucose transport. As a result, length of contact by released glucose and thus response is similar with varying duodenal loads of most dietary carbohydrates.

Fats. It is now known that suppression of food intakes by long-chain or medium-chain triglycerides is completely blocked if lipolysis is inhibited (27). Therefore, we studied the efficacies of fatty acids, and we confirmed the observation of Yox and Ritter (41) that octanoic acid did not inhibit sham feeding even when given in amounts isocaloric with potent doses of dodecanoic acid (Table 1), nor did decanoic acid inhibit sham feeding. On the other hand, dodecanoic acid was about as potent on a molar basis as oleic acid (Figs. 1 and 4). Because rates of absorption of fatty acids across mucosal unstirred water layers decrease dramatically as fatty acid chain length is elongated, it is likely that rates of absorption and subsequent metabolism of C-8 and C-10 must have been completed much more quickly than that of C-12; whereas C-18 is known to be absorbed about one-half as fast as C-12, yet neither C-8...
nor C-10 were satiating, but C-12 and C-18 were about equally effective.

All of these fatty acids are metabolized, yet in the amounts given here, only luminal C-12 and C-18 suppressed food intake. This observation argues strongly that the suppression was mediated at the intestine by a sensory system sensitive to fatty acids longer than 10 carbons. Indeed, caval, portal, or systemic venous injections of fats did not suppress feeding as much as intraduodenal instillations of the same loads of fatty acid (14, 34, 37).

The actions of the fatty acids are further complicated by differences in their postabsorptive transports. Absorbed fatty acids of chain lengths ≦10 carbons are water-soluble enough to be transported from the absorptive cell to the systemic circulation via the portal blood (19), but absorbed fatty acids ≧12 carbons are reesterified into triglycerides in the absorptive cell, packaged in chylomicrons [surrounded by hydrophilic apolipoproteins (Apo)], and transported to the systemic circulation via the lymph. Lipoprotein synthesis and secretion by the absorptive cell is induced by the absorption of longer-chain fatty acids. One of these lipoproteins, Apo A-IV is uniquely synthesized by the intestinal absorptive cell and is itself capable of crossing the blood-brain barrier and directly triggering satiety centers in the hypothalamus (a fuller discussion appears in Ref. 27). Furthermore, blocking the transport of chylomicrons and, concomitantly, the secretion of Apo A-IV with \( \text{l}-\text{pluronic acid-81} \) abolished satiety responses in rats to intragastrically instilled corn oil (27). In view of these various observations, it is possible that (equi)potencies of C-12 and C-18 but the impotencies of C-8 and C-10 derive somehow from mediation by Apo A-IV of the intestinal satiety signals from lipolytic products.

Amino acids. Among amino acids tested, potency was limited to tryptophan and phenylalanine. Both \( \text{l}-\text{phenylalanine (41) and l-tryptophan (5)} \) have been previously shown during intestinal infusions to potentially suppress nutrient intakes. Our data indicated that phenylalanine or the phenylalanine-tryptophan (Fig. 3) did inhibit sham feeding, but the effects were weak and only significant during our midgut perfusions. In the naturally feeding rats, the mixture of phenylalanine plus tryptophan potently inhibited food intake (Fig. 4) more so than in the sham-fed animals.

Temporality

We examined the persistence of suppression of food intake after perfusions were stopped in tethered rats (Table 6 and Fig. 7). The experiments indicated that 7.2 mmol of maltose given over 1.5 h immediately before feeding had virtually no carryover effect. Dodecanoate (1.44 mmol) continued to suppress intake for \( \approx 2 \) h, whereas oleate continued to suppress intake for \( \approx 3 \) h after stopping it. It is known (28) that maltose is rapidly absorbed (as rapidly as glucose) and transported into portal blood so that it does not accumulate in gut lumen or wall. Its lack of persistence within intestinal lumen or wall corresponded with a lack of sustained suppression of food intake after stopping the perfusion.

In contrast to maltose, fatty acids are slowly absorbed; for example, we have estimated that dogs absorbed oleate about one-tenth to one-hundredth as fast as glucose on a molar basis (28). Therefore, it is likely that a significant amount of infused oleate persisted in the intestinal lumen for some time after stopping the infusions. In addition, reesterification and transport of absorbed fatty acids into lymph is slow; it begins after \( \approx 30 \) min and continues at peak rates for up to \( 2 \) h after stopping intestinal infusions (14, 27). However, suppression of food intakes by luminal fats starts within 10 min of infusion into duodenum (14). The prompt suppression of intake after starting intestinal perfusions (Table 6) and then a sustained suppression of food intake for \( \approx 2 \) h but \( \approx 3 \) h after stopping the perfusions of oleate (Fig. 7) is consistent with these several observations. The somewhat shorter (\( \approx 1 \) h but \( \approx 2 \) h) persistence of suppression from C-12 can be well explained by the facts that dodecanoate is absorbed about two times as fast as oleate but thereafter is transported into lymph as slowly as oleate. In this scenario of persisting suppression by fatty acid in lumen and/or enterocyte, the gastrointestinal locus is a signalling way station from which triglyceride is slowly exported into lymph and ultimately blood, where it no longer suppresses food intakes (14, 34, 37).

Amino acids are actively transported at about one-half the rate of glucose but considerably faster than fatty acids. Yet, the 4.3 mmol of phenylalanine plus tryptophan exhibited a peculiar time course for suppression; an initially smaller difference between intakes after amino acid perfusions vs. control over the first \( 1-1.5 \) h continued to widen over 1.5–3 h after stopping the perfusion (Table 6 and Fig. 7A). Because all four perfusates in Table 6 had suppressed food intakes in these animals to 8–9 g when instilled over the entire 3 h of intake (data not shown), the smaller suppression of 3-h intakes (to \( \approx 12 \) g) after only 1.5 h of perfusion with glucose, methylglucose, or dodecanoate indicated that suppression from these three perfusates was transitory. By contrast, phenylalanine plus tryptophan suppressed intakes to \( \approx 9 \) g whether infused for the initial 1.5 h or for the full 3 h of the feeding period. It is known that some dietary tryptophan crosses the blood-brain barrier after absorption from the diet to be taken up by the brain and converted to serotonin. In previous experiments in rats, the concentration of tryptophan and of serotonin in brain (11) had begun to rise by 1 h after ingestion of tryptophan-containing food and continued to increase for up to 2 h (no measurements reported thereafter). Increased levels of serotonin at synapses in the hypothalamus enhance satiety (4). The increasing suppression of food intake after phenylalanine plus tryptophan were stopped in our experiments (Table 6 and Fig. 7), consistent with increased satiety from an accumulation of tryptophan and serotonin in the brain. A lower efficacy during the first 1.5 h of perfusion but a higher efficacy later (Table 6) could well have accounted for the greater effect of these amino
acids over the 3 h of perfusion in the tethered rats compared with the sham-fed animals (the latter were perfused and observed for only 90 min).

Sensitivities of Intestinal Regions

At the start of these studies, there was little and inherently conflicting information on the distributions of nutrient-driven satiety feedback along intestine. Welch et al. (37,38) had demonstrated in humans and Glick (13) in rats that similar satieties were evoked by separate perfusions of jejunum and of ileum with emulsified corn oil or glucose, respectively, suggesting that feedback was similar from these two regions. By contrast, from his observations after a variety of intestinal transpositions and crossed intestinal perfusions, Koopmans (20) concluded that, in the rat, nutrient-driven satiety from ileum was many times more intense than very weak, almost absent feedback from similar lengths of jejunum. In dogs (9,26), we suppressed food intakes by promoting malabsorption via diversion of biliary or pancreatic secretions that spread digesting nutrients farther and abnormally into ileum. Thus we reasoned that such abnormal spread into ileum promoted satiety either (1) by triggering more potent feedback per length of ileum (Koopmans’ idea) or (2) by summatting approximately equal satiety responses per length of jejunum with those from ileum (Welch’s idea).

On the basis of similar slopes from dose-response curves during infusions of digestive products into duodenum or midgut (Figs. 1–5), we conclude that ileal and jejunal responsiveness for satiety is about equal in rats for digestive products of the three macronutrients, a conclusion quite similar to that derived from more limited observation by Glick (13). Almost concurrently, Woltman and Reidelberger (39) also studied dose-responsive reductions of food intakes during infusions of various nutrients into duodenum or terminal ileum of naturally and continuously feeding rats. During the 2 h of actual perfusion (the closest parallel between their design and ours) there were no substantial differences between dose responses after duodenal vs. ileal glucose. With oleate, by contrast, Woltman observed significantly more dose-responsive inhibition of food intakes during duodenal than during ileal perfusions, just as we observed a significantly steeper dose response to duodenal compared with ileal oleate (Fig. 4) in sham- and naturally fed animals. Finally, we found that removing most of the ileum did not alter the slopes of dose responses but shifted the dose response, significantly so with oleate-monolein (Fig. 6).

Analyses of effluents in other experiments (28) indicated that, during duodenal infusion of 0.96 mmol/h of oleate, fatty acid was incompletely absorbed by small bowel, and some passed into colon. This result corresponded with the propensity of duodenal loads of oleate (sometimes at the 0.48 and nearly always at the 0.96 mmol/h for 3 h) to induce diarrhea in the naturally fed rats and for both loads of oleate to always induce diarrhea on 3 h infusion into midgut. The various observations suggest that considerable lengths of intact intestine were exposed to still unabsorbed fatty acids after the higher loads. We were therefore surprised to observe that removing nearly one-half of the distal small intestine (a procedure that greatly increased the frequency of diarrheas after perfusion of all four nutrients) did not much alter slopes of the dose responses to duodenal instillations, although removing ileum did significantly decrease suppression from both tested doses of oleate-monolein (Fig. 6). However, we found that the colon also was capable of signalling satiety (Table 5). Because the colon lacks glucose transporters (6) and mechanisms to export chylomicra and Apo A-IV, it is likely that other pathways from colon suppressed food intake (like release of PYY or absorption of bacterial conversion products). Whatever should prove to be the mechanisms for colonic suppression, we speculate that, in the unresected animals, satiety was signalled with increasing duodenal loads from jejunum plus increasingly ileum but during infusions of increasing loads at midgut from ileum plus increasingly colon. In the ileal resected animals, dose responsiveness to duodenal infusions was preserved by recruitment of sensors in jejunum plus colon. In this regard, it is of interest that Woltman and Reidelberger’s (39) ileal infusion site was only 14 cm (out of a small intestinal length of 110 cm) from the cecum. We speculate that their dose responses to ileal infusions were generated mostly by sensors in the colon.

These recent observations would seem entirely to refute Koopmans’ (20) findings. However, both we and Woltman and Reidelberger (39) studied infused digestive products, whereas Koopmans observed intakes after altering intestinal exposures to naturally digestible chyme. Differences in experimental design raise two possibilities that could resolve the conflicting conclusions. 1) It is conceivable that some partially digested polymeric foods trigger different sensors with differing distributions than those excited by our monomeric products. Although there was some increase in efficacy of midintestinal Polycose over maltose (Fig. 5), the difference was not sufficient to account for Koopmans’ observations. Furthermore, experiments with acarbose (Table 4) indicated that Polycose acted after hydrolysis, not through sensors directed at specific polysaccharides. 2) More likely, however, the ileojejunal transpositions that Koopmans utilized resulted in a premature luminal disappearance of bile salts through their active ileal absorption and also the release by ileal fat of “pancreotones,” like peptide YY (35) known to potentiate inhibit pancreatic secretion in the rat. If so, digestion of chyme would have been slowed, and the release of digestive products spread along much longer lengths of bowel to give a higher suppression (28), much as similarly displaced digestions produced augmented satieties in our dogs (9,26).

Reduction Ratios

Reduction ratios >1.00 seem to contradict observations that animals accurately adjust their caloric in-
takes either to appropriately increase consumption of food diluted with noncaloric substances (1, 3, 17) or to reduce caloric intakes in a calorie for calorie compensation to gastric instillation of nutrient calories in “pre-meals” just before the feeding period (24, 27). Nevertheless, it is well known from radiographic assessment of gastric emptying that accurate compensations to pre-meals arose when only a portion of the premeal had entered small intestine (12, 25), so, in fact, even this simple analysis of gastrointestinal events suggests that reduction ratios from intestinal feedback must have exceeded 1.00 initially. In a subsequent study (27), we confirmed that gastric emptying of lipid calories is not uniform over time in the rat but is initially rapid and that during this initially rapid emptying of oil pre-meals, fatty acids released into small intestine had reduction ratios from 2.7 to 4.3. Reduction ratios fell in subsequent postcibal hours. It seems, therefore, that 1:1 overall caloric compensations (which imply reduction ratios of 1.00) over an extended period of feeding reflect an average over time of widely fluctuating levels of intestinal feedback.

We are grateful to Kent Lloyd and Stanley Ashley for schooling us in various surgeries, and we wish to thank Donald J. Ehn and Margaret Baker for technical assistance. This work was supported by research funds from the Department of Veterans Affairs.

Address for reprint requests: J. H. Meyer, Rm. 105, Bldg. 115, West Los Angeles VAMC, 11301 Wilshire Blvd., Los Angeles, CA 90073.

Received 23 August 1996; accepted in final form 23 June 1998.

REFERENCES