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# Comparison Between some Digestive Processes after Eating and Gastric Loading in Rats

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MOLINA, F., T. THIEL, J. A. DEUTSCH AND A. PUERTO. *Comparison between some digestive processes after eating and gastric loading in rats.* PHARMAC. BIOCHEM. BEHAV. 7(4) 347–350, 1977. – Physiological and biochemical differences were found between normal and intragastric feeding in the rat. Milk ingested orally remained in the stomach significantly longer than milk ingested via a chronic intragastric fistula. Milk samples were removed from the stomach at intervals of 5, 20 and 45 min after ingestion and it was found that milk which had been ingested via intragastric fistula underwent substantially less lipolysis than did milk which had been ingested orally. These results are discussed in relation to the aversive and rewarding effects obtained by injections of edible nutrients into the stomachs of rats.

Aversion  Reward  Cephalic phase  Feeding  Lipolysis  Gastric emptying

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RATS develop a very strong tendency to avoid intragastric injections of nutrients which otherwise they will ingest voluntarily (e.g., sesame oil) [9]. The cause of such an aversion is unknown. Further, hungry rats are unable to recognize intragastric injections of whole milk as a reward, unless such milk has been previously digested by a donor rat [25,26]. We have hypothesized [27] that the explanation of this phenomenon could be due to what Pavlov [24] called the psychic stimulus (effect) or cephalic phase, as it has been named by others [12, 21, 30, 33]. The cephalic phase can be described, in general, as the stimulation of secretions in the alimentary canal by stimuli arising from the brain or by agents acting upon the central nervous system [35]. It was about seventy-five years ago when Pavlov observed that the flow of gastric juice was triggered mainly by the expectation and sensory stimulation of food, and only to a lesser extent by the food itself inside of the stomach. It would seem probable from the above observations that an abnormal state of affairs arises when food is loaded directly into the stomach without the stimulus or act of eating.

Further, if there is less HCl to mix with the injected food than if such food were normally ingested, there should be a lower $H^+$ ion concentration of material leaving the stomach. This should lead to a lesser secretion of secretin and cholecystokinin when this material enters the duodenum. It is known that secretin and cholecystokinin decrease the rate of gastric emptying [4,15], and so a higher pH of material arriving in the duodenum should lead to a higher rate of gastric emptying [13,17]. Essentially, it would be predicted that a disruption of the normal digestive processes through slower enzymatic breakdown and hastened gastric emptying would occur if food arrived in the stomach without the stimuli accompanying normal food ingestion.

The most frequent tool for the study of the effects of the cephalic phase has been the amount of secretion of hydrochloric acid [19,35] or pepsin [23]. Another component of gastric juice is lipase [5,16]. This enzyme has been shown to be present in different species [2, 3, 29, 32] and its output is highly correlated with that of HCl, both being controlled by the same vagal stimuli [7,32].

## EXPERIMENT 1

In this experiment, we predicted that the rate of lipolysis which fresh milk undergoes in the stomach will be different depending on whether milk arrived at the upper gastrointestinal tract after normal feeding behavior or after intragastric injection. After intragastric injection food is present in the stomach for the same intervals of time but the cephalic phase has been bypassed. It was expected that the absence of the cephalic phase would decrease the breakdown of fats into free fatty acids and this is indeed what we have found.

### *Method*

*Animals.* Animals were eight male Sprague-Dawley (Simonsen) rats, weighing 300–400 g at the time of surgery. Intragastric fistulae were implanted according to a procedure described elsewhere [8]. Animals were allowed to recover for one week. During this period solid food and water were available ad lib. Following recovery rats were trained to eat liquid nutrient only (evaporated whole milk, Carnation Co.).

*Experimental procedure.* This experiment manipulates two variables: First, rats were either allowed to drink milk or were intubated. Second, the amount of time was varied between drinking (or intubation) and aspiration of stomach

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contents. Five, twenty and forty-five min delays were employed. Drinking and intubation conditions were run on alternate days in each rat; intubation days always following drinking days. For each rat the amount of milk intubated and the rate of intubation were always the same as the amount drunk and the rate of drinking of the previous day (see Table 1).

TABLE 1

MEAN VALUES FOR THE RATE AND VOLUME OF MILK INTUBATION

| | 5 min | 20 min | 45 min |
|---|---|---|---|
| Mean of time | 8 min | 8 min 43 sec | 8 min 48 sec |
| Mean of volume | 16 cc | 19.4 cc | 19.2 cc |

Values are equal to rate and volume of oral milk consumption measured on the previous day.

A total of 48 5 cc extractions were obtained. Each one of the samples to be analyzed consisted of two of these 5 cc extractions. Twenty-four samples were thereby analyzed; four in each of the experimental conditions.

The samples were immediately analyzed under single-blind conditions. The fat portion of each sample was extracted using the Rose-Gottlieb Method (AOAC Method 15029) as specified for evaporated milk [20]. The free fatty acid (FFA) proportion was then determined by the standard method [14].

### Results

Results are shown in Fig. 1 and Table 2. The percentage of free fatty acids is plotted as a function of time for both conditions. There is a significant difference between the two conditions (milk drunk or injected): 5 min interval ($t$ = 10.47, $p<0.01$); 20 min interval ($t$ = 10.97, $p<0.01$); 45 min interval ($t$ = 14.62, $p<0.01$). Milk placed directly into the stomach undergoes much less lipolysis in the same amount of time than milk drunk in a normal manner. Intragastric injections (bypassing the effect of the cephalic phase) produce an abnormal enzymatic breakdown of fats. The gastric phase is unable to overcome such abnormalities even after 45 min.

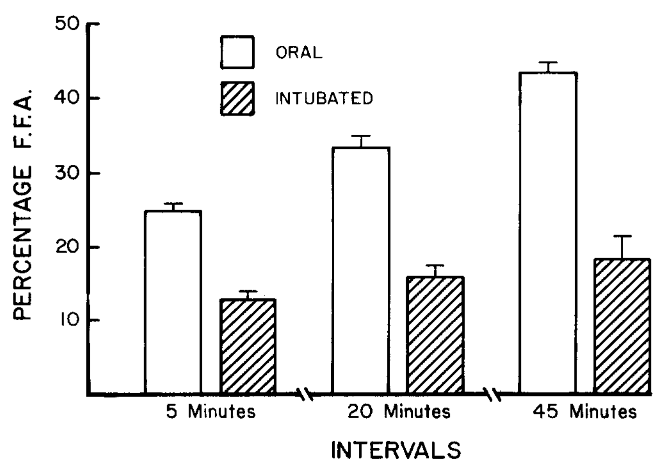


FIG. 1. Percentage of free fatty acids as a function of digestion time following oral consumption or intragastric injection of milk.

TABLE 2

MEAN PERCENTAGE (± SEM) OF FREE FATTY ACIDS AS A FUNCTION OF DIGESTION TIME FOLLOWING ORAL CONSUMPTION OR INTRAGASTRIC INJECTION OF MILK.

| Time | % FFA | SEM | % FFA after | SEM |
|---|---|---|---|---|
| After 5 min | 24.7 | 0.72 | 11.8 | 1.04 |
| After 20 min | 33.0 | 1.62 | 13.2 | 1.25 |
| After 45 min | 43.3 | 1.69 | 18.5 | 2.97 |

## EXPERIMENT 2

It is known that an increase in the rate of gastric emptying produces distressing effects in patients (e.g., dumping syndrome) [28]. These changes in gastric emptying have been suggested to be the consequence of abnormal digestive processes such as those taking place after vagotomy [18], absence of salivary secretion [22] or achlorhydria [13].

In Experiment 1 we have shown that the absence of the cephalic phase due to direct intragastric injections produces an abnormal enzymatic breakdown of food. The goal of Experiment 2 was to find out if normal and intragastric feeding empty from the stomach at different rates. This could help to understand some of the reasons of the aversion reactions it produces [9].

### Method

*Animals.* Animals were 18 male Sprague-Dawley (Simonsen) rats of the same description as in Experiment 1. The surgery and the recovery period were also exactly the same. Rats were trained to drink a 16.2% dextrin solution (General Biochemicals Inc., Chagrin Falls, OH) until satiated. The training period lasted three days.

*Experimental procedure.* After recovery, animals were divided randomly into six groups (three animals each). On the day of the experiment, half of the rats (three groups were given 15 cc of dextrin solution to drink while the other half (the remaining three groups) were injected by fistula at the same rate and with the same amount of dextrin. One group from the oral condition and another from the intubated condition were sacrificed at the end of each of the following intervals; 5 min, 45 min and 150 min: each animal was anesthesized and the abdominal cavity opened. The stomach was gently lifted outside the body cavity; the esophagus and the pylorus were clamped and tied. Immediately after, the stomach was removed and its volume measured.

### Results

At each individual interval the volume of gastric contents remaining in the stomach was significantly greater in every animal of the oral condition than in the intubated condition for each time interval. (Mann-Whitney U, $p<0.01$). These results show that food which arrives at the stomach after bypassing the cephalic phase has a greater rate of gastric emptying.

A comparison of mean stomach volume for both conditions at different intervals is shown in Fig. 2 and Table 3.

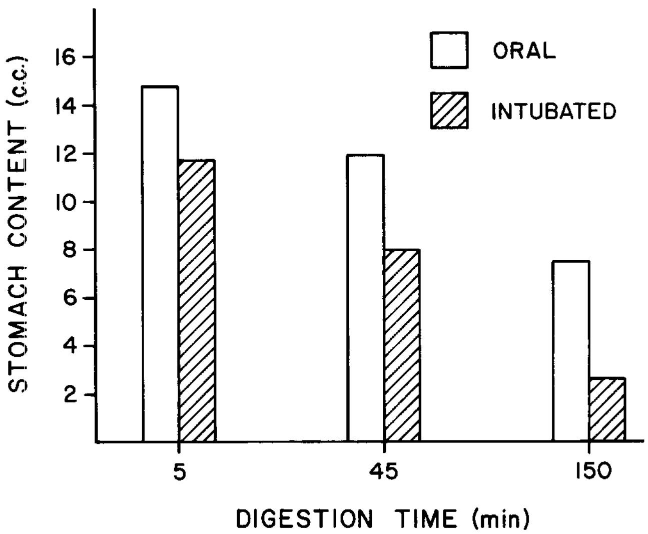


FIG. 2. Volume of stomach content as a function of digestion time following oral consumption or intragastric intubation of millk.

TABLE 3

MEAN VOLUME OF STOMACH CONTENTS AS A FUNCTION OF TIME FOLLOWING ORAL CONSUMPTION OR INTRAGASTRIC INTUBATION OF A 16.2% DEXTRIN SOLUTION

| | 5 min | 45 min | 150 min |
|---|---|---|---|
| Oral | 13.8 cc | 11.7 cc | 7.5 cc |
| Intubated | 11.6 cc | 8.0 cc | 2.6 cc |

## DISCUSSION

The primary conclusion from these experiments is that food intragastrically intubated does not evoke the same physiological and biochemical mechanisms which take place during normal feeding behavior.

In Experiment 1 it is shown that the rate at which lipolysis of milk fat proceeds in the stomach is much faster when the milk is normally drunk. Intragastric injections of the same volume and at the same rate animals usually drink lead to a slower overall rate of lipolysis. These results agree with those reported by Clark, Brause and Holt [6]. They showed no effect of gastric lipase during the first 15 min after intragastric intubation of fat in pyloric ligated rats. Furthermore, our data suggest that with food intragastrically injected, low lipase activity occurs even after 45 min; this is not the case during normal feeding behavior. Thus, it can be concluded the cephalic phase is necessary for normal lipolytic activity.

We do not know if this enzymatic action is due to gastric or duodenal lipase. However, several lines of evidence argue against a lipase reflux from the duodenum. It has been shown [10,11] that this mechanism is very unusual in healthy subjects. Furthermore, pancreatic lipase is active at pH of 6–8 while the activity of gastric lipase occurs at pH of 3–4 [3,7].

The Experiment 2 shows that intragastric injections of nutrients lead to a much faster rate of gastric emptying than when the same nutrients are normally drunk. This premature entering of food into the duodenum has been reported to produce markedly distressing effects [28]. Gastric emptying is very sensitive to a series of digestive abnormalities in the processes of digestion. Malhotra [22] has shown that saliva decreases the rate of gastric emptying, which he attributes to the action of salivary amylase. However, even with normal salivary secretion, an increased gastric emptying takes place in patients suffering from achlorhydria [13] or as a result of vagotomy in experimental animals fed with liquid diet [18,31]. Similar results are obtained when abnormal lipolytic breakdown occurs, as can be seen if Experiments 1 and 2 are taken together. But it is known that salivary and gastric secretions are resultant components of the cephalic phase, which is mediated in part by the vagus nerve. Thus, the cephalic phase seems to be very much related to gastric emptying. When one or more of its components are absent, the rate of emptying is significantly increased.

In conclusion, these experiments suggest that a series of digestive abnormalities are produced by food intragastrically injected [1,34]. These abnormalities could be one of the explanations of the aversive reactions found in rats with the intubation of otherwise palatable nutrients [9]. On the other hand, a rewarding effect is found when the food injected has been previously digested by donor animals. There the cephalic phase has occurred in the donor rat, and the food intragastrically injected into the recipient has undergone the biochemical processing which takes place during normal feeding behavior [26].

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