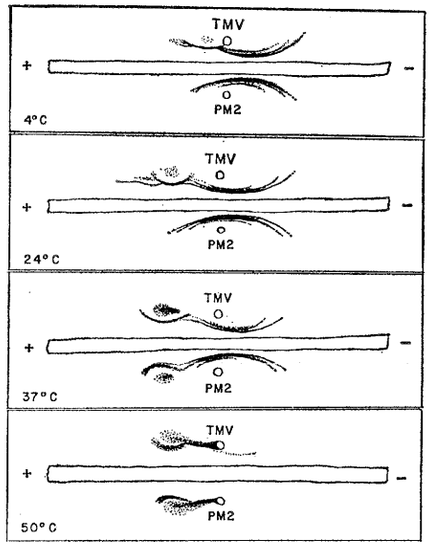

Fig. 2. Immunoelectrophoretic analysis of PM2 and TMV protein conducted at different temperatures. Antiserum to TMV protein was used to develop the precipitation patterns.

erties of both the wild-type TMV protein and the mutant PM2 protein. Below 37°C, TMV protein and PM2 are easily distinguishable. Apparently, a number of aggregates with greater electrophoretic mobility are present in the wild type at the lower temperatures. No counterpart to these antigens is seen in PM2. Raising the temperature to 37°C or above has the striking effect of making the two antigen preparations appear alike. This is in agreement with the results obtained by Ouchterlony analysis.

The defect associated with PM2 resides in the replacement of threonine by isoleucine in position 28 and in the replacement of glutamic acid by aspartic acid in position 95 of the polypeptide chain (*8*). Whether only one or both amino acid replacements are necessary for the expression of defectiveness is unknown. However, these data cast additional light on the consequences of the mutational event. The two amino acid replacements in PM2 protein are probably instrumental in preventing the subunits from polymerizing to the same degree as wild-type TMV protein at temperatures below 37°C. At 37°C and above, however, polymerization of PM2 appears normal by immunological criteria; this suggests that at least the conformation of the polypeptide at the antigenic surface of both TMV and PM2 is now the same. It is important to determine whether the defect in PM2 can be repaired by elevating the temperature so that the protein would polymerize with RNA and would aggregate into rodlike structures. Preliminary electron micrographs of PM2 protein incubated at 45°C indicate formation of somewhat more compact structures than the loose coils reported earlier (*2*).

IRVING RAPPAPORT
Department of Microbiology, New York Medical College, New York
MILTON ZAITLIN
Department of Agricultural Biochemistry, University of Arizona, Tucson

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Stretch Receptors in the Foregut of the Blowfly

Abstract. *Two bipolar neurons are located in a nerve branch connecting the recurrent nerve and foregut of the blowfly* Phormia regina. *Spike activity accompanying peristalsis or controlled enlargement of the foregut region is recorded from two cells in the recurrent nerve. The spikes are abolished by section of the nerve branch connecting recurrent nerve and foregut. It is concluded that the two neurons are the foregut stretch receptors predicted from results of behavioral experiments and vital to the regulation of feeding by the fly.*

A homeostatic mechanism controls the feeding behavior of the blowfly *Phormia regina* such that, in the presence of excess food, a constant amount is ingested per day (*1*). The essential element of the homeostatic mechanism is a negative feedback relationship whereby a consequence of feeding, filling the gut, reduces the probability of further feeding (*2*). Filling the gut activates receptors in the gut and body wall; the activity of these receptors inhibits in the brain the input from the external chemoreceptors which elicits feeding (*2, 3*).

The receptors in the gut wall are located in the region of the foregut (*2, 3*). Filling the other two gut segments, the hindgut and midgut, by enema has no effect on behavioral sensitivity to gustatory stimuli (*2*). Behavioral data indicate that the foregut receptors are stretch receptors (*4*). The foregut receptors have now been localized anatomically, and some of their electrophysiological characteristics have been determined.

The foregut-recurrent nerve complex in *Phormia* was dissected out; in some blowflies it was previously stained with leuco-methylene blue by the method of Zacharuk (*5*). Other preparations of freshly isolated recurrent nerve were stained with Giemsa stain for 15 minutes. These were examined immediately after isolation and vital staining. Suction electrodes were used for electrophysiological recording. The electrode assembly consisted of a micropipette coupled directly to a 1-ml syringe with a Tuohy adapter. A silver wire was inserted into the pipette to its tip and brought to the outside through the rubber gasket of the Tuohy adapter. The orifice of the electrode was adjusted so that a loop of nerve formed a snug fit when drawn into the electrode. Recording was differential between the electrode and the surrounding saline and was capacitatively coupled.

Controlled enlargement of the gut lumen was accomplished with an electrically driven microsyringe. A micropipette was converted into a microsyringe by filling it with fluid and inserting a close-fitting insect pin into its shank to act as a plunger. The insect pin was coupled to the cone of a loudspeaker so that short electrical pulses to the speaker were converted to short pulses of fluid from the tip of the micropipette. A gut segment was ligatured over the tip of the micropipette and was stretched by the hydraulic pulses.

A fine nerve branch arises from the ventral surface of the recurrent nerve and connects with the anterior region of the foregut. If this branch is cut at its termination on the foregut and is vitally stained with Giemsa stain for 15 minutes, two bipolar neurons can be seen within it (Fig. 1). These cells are typical insect neurons

having large nuclei and prominent nucleoli.

A fly was dissected so that the recurrent nerve anterior to the brain was exposed. A loop of the nerve was drawn into a suction electrode. Under these conditions bursts of activity are usually recorded. If the foregut is observed while the spike activity is monitored aurally, the bursts of activity are seen to be in synchrony with spontaneous expansions of the gut lumen. The bursts are composed of spike trains from two cells; they continued unabated after the connection of the recurrent nerve to the brain was severed at the frontal ganglion. If the gut becomes quiescent in an expanded state, the activity of two units each firing at a steady rate can be recorded (Fig. 2A). The responses of the two cells in the record shown differ slightly in spike form and can readily be distinguished on close inspection. One unit in Fig. 2A is firing at 18 impulses per second, while the other is firing at 13 impulses per second. Gentle stretching of the nerve branch which connects the recurrent nerve and foregut sometimes causes one unit to cease its activity (Fig. 2B). The unit remaining continues to fire regularly until the nerve branch connecting the recurrent nerve and foregut is completely severed, following which no responses to spontaneous gut movements are recorded.

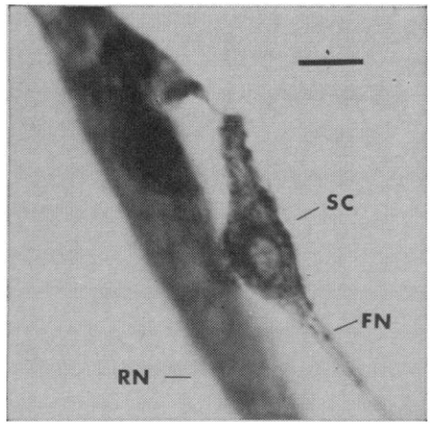

Fig. 1. Bipolar neuron stained vitally with Giemsa stain and located in the nerve branch connecting the recurrent nerve and foregut. The second neuron contained in the branch is out of focus and beneath the one shown. The connection of nerve branch to recurrent nerve was injured in dissection. *RN*, recurrent nerve; *SC*, sense cell; *FN*, nerve to foregut. Calibration line measures 10 μ.

To bring the response under greater control, a speaker-activated microsyringe was introduced into the gut lumen at the crop and threaded up the crop duct to the foregut. A ligature of raw silk from a cecropia cocoon anchored the gut to the pipette tip. A second ligature around the esophagus just anterior to the foregut limited the gut segment expanded by the fluid pulses to the foregut region. Recordings from the recurrent nerve were made while the foregut lumen was expanded for various lengths of time and to various degrees. In all cases the recurrent nerve was severed at the frontal ganglion.

Latency of the response of one of the two sense cells to controlled enlargement of the gut lumen was about 1 to 2 msec (Fig. 2C). Following an initial response decrement probably due to muscular accommodation, a steady rate of firing is maintained. The occasional spikes seen when the gut is allowed to slacken result from spontaneous movements of the gut musculature. If a constant stretch is applied for long periods, a sustained and uniform rate of firing is produced (Fig. 2D). This steady response is maintained apparently indefinitely.

The existence of foregut stretch receptors hypothesized on the basis of behavioral experiments (*2*, *4*) has

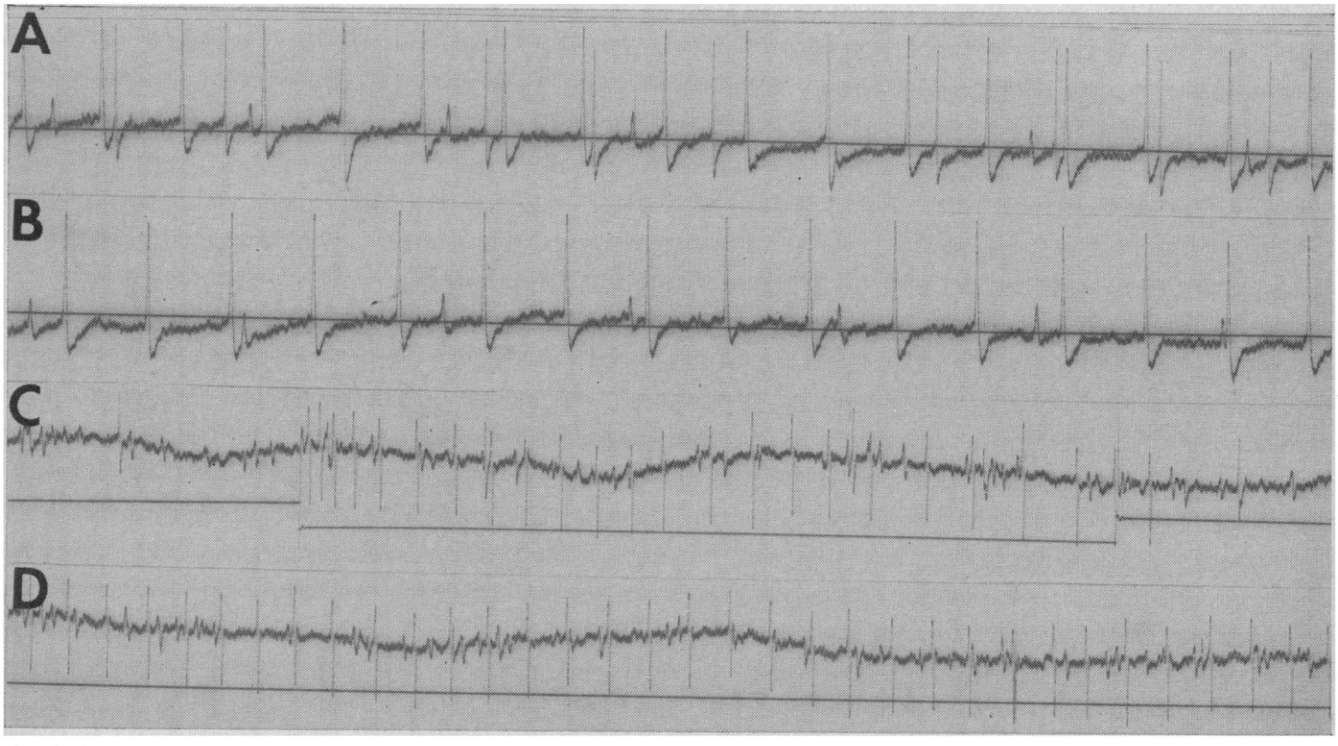

Fig. 2. Sensory impulses recorded from the recurrent nerve in response to enlargements of the foregut lumen. (A) Gut quiescent in expanded state. (B) Recording after nerve branch to foregut slightly stretched. (C) Gut lumen expanded with hydraulic pulse; downward deflection of lower trace marks stimulus on; upward deflection marks stimulus off, pulse length 600 msec. (D) Response to sustained stretch.

now been fully confirmed anatomically and electrophysiologically. These receptors send information to the brain regarding the extent and duration of peristalsis in the foregut, which is also a measure of the fullness of the crop as the crop contents pass through the foregut on the way to the midgut for digestion (*6*). On the basis of behavioral experiments, the action in the brain of the stretch receptor input is hypothesized to be inhibition of external chemoreceptor input (*1*). If the fly is deprived of the input from the foregut receptors by cutting of the recurrent nerve, feeding behavior is not inhibited in the normal manner, and hyperphagia results (*3*).

ALAN GELPERIN
Department of Biology, Tufts University, Medford, Massachusetts

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Enterogastrone Inhibits Eating by Fasted Mice

Abstract. *In mice fasted for 17 hours, administration of enterogastrone purified from hog duodenum reduced the food intake. This effect was greatest during the first 30 minutes, but the cumulative reduction continued for at least 4 hours. Other peptides prepared from hog duodenum or colon, as well as glucagon, secretin, glucose, and bovine serum albumin, were ineffective.*

The mechanisms controlling food intake are many and their interrelations are complex (*1–4*). Among the factors suggested are sensations from the digestive tract associated with eating and the presence of food in the stomach and the intestines (*3, 4*). Gastric contractions appear to be a common element of the hunger complex (*2*). The hormone enterogastrone, derived from the small intestine, is known to reduce gastric secretion and gastric motility (*5*), and so could be associated with inhibition of eating (*4*). Grossman (*4*) and Janowitz and Grossman (*6*) tried to elicit release of enterogastrone, and thus inhibit gastric contractions, by prefeeding dogs with sucrose or cream. Small amounts of these nutrients did not reduce the subsequent consumption of food, but larger amounts did. They concluded that enterogastrone may not play a role in regulation of food intake.

Availability of purified enterogastrone (*7*) prompted us to reexamine the role of enterogastrone in the regulation of food intake. Enterogastrone and other peptides were prepared from extracts of hog duodenum. The purification of enterogastrone and its effects on gastric secretion will be detailed elsewhere (*7*).

We trained female mice of the White Swiss strain (25 to 30 g in body weight) to eat a liquid diet consisting of 7.4 percent protein, 11.6 percent carbohydrate, and 2.1 percent fat. They were fasted for 17 hours, but water was always provided before and during the test period to eliminate the effects of thirst and dehydration. The mice were then injected intravenously or subcutaneously with the test substances; control mice were injected with saline or carrier vehicle. Ten to 15 minutes later, food, in a special volumetric feeder, was placed in their cages and food consumption was measured after 30, 60, 120, and 240 minutes. The results are presented as cumulative intake by volume for each period in comparison with those of control groups of mice. At least three experiments were carried out with each of the substances tested, with ten mice per group in each experiment.

The results (Table 1) indicate that a

Table 1. Effects of enterogastrone and other substances on food consumption by fasted mice. Each value is the mean for three experiments; each experiment used ten mice. The carrier contained 16 percent gelatin and 0.5 percent phenol. Abbreviations: i.v., intravenous; Ent 1, enterogastrone batch 1; NS, not significant; Ent 2, enterogastrone batch 2; s.c., subcutaneous; Duod 1, duodenal peptide batch 1; Duod 2, duodenal peptide batch 2; Colo, colonic fraction; Secr, secretin; Gla, glucagon; Bov, bovine serum albumin; Glo, glucose; i.p., intraperitoneal.

Time (min)	Cumulative intake per mouse (ml, ± S.E.)		*P*	Cumulative intake per mouse (ml, ± S.E.)		*P*
	Control	Injected		Control	Injected	
	Saline i.v.	*Ent 1 i.v. (0.1 mg)*		*Saline i.v.*	*Ent 1 i.v. (1 mg)*	
30	1.5 ± 0.1	0.7 ± 0.15	.01	2.5 ± 0.1	0.2 ± 0.0	.001
60	2.4 ± 0.06	1.6 ± 0.2	.02	3.1 ± 0.06	.9 ± 0.2	.001
120	3.4 ± 0.1	2.7 ± 0.26	NS	3.8 ± 0.12	2.4 ± 0.04	.001
180	4.3 ± 0.07	3.6 ± 0.2	NS	5.0 ± 0.12	3.7 ± 0.16	.001
240	4.9 ± 0.22	4.2 ± 0.2	NS	5.9 ± 0.2	5.0 ± 0.14	.05
	Saline i.v.	*Ent 2 i.v. (1 mg)*		*Carrier s.c.*	*Ent 1 s.c., carrier (1 mg)*	
30	1.3 ± 0.1	0.00 ± 0	.001	1.6 ± 0.2	0.7 ± 0.1	.02
60	1.7 ± 0.04	.4 ± 0.0	.001	2.2 ± 0.3	1.0 ± 0.2	.05
120	2.8 ± 0.1	1.6 ± 0.3	.001	2.9 ± 0.3	1.6 ± 0.2	.05
180	3.7 ± 0.2	2.7 ± 0.04	.01	4.0 ± 0.3	2.5 ± 0.3	.05
240	5.0 ± 0.3	3.6 ± 0.1	.01	5.0 ± 0.3	3.5 ± 0.4	.05
	Saline i.v.	*Duod 1 i.v. (1 mg)*		*Saline i.v.*	*Duod 2 i.v. (1 mg)*	
30	2.4 ± 0.1	1.0 ± 0.3	.005	1.7 ± 0.11	1.3 ± 0.04	.05
60	2.7 ± 0.2	1.8 ± 0.4	NS	2.1 ± 0.1	1.8 ± 0.1	NS
120	3.3 ± 0.3	2.4 ± 0.6	NS	3.1 ± 0.15	2.6 ± 0.2	NS
180	3.8 ± 0.5	2.8 ± 0.4	NS	3.9 ± 0.13	3.8 ± 0.15	NS
240	5.2 ± 0.4	4.4 ± 0.7	NS	5.1 ± 0.1	5.2 ± 0.2	NS
	Saline i.v.	*Colo i.v. (1 mg)*		*Saline i.v.*	*Secr i.v. (10 μg)*	
30	2.6 ± 0.2	2.0 ± 0.15	NS	2.1 ± 0.2	2.2 ± 0.1	NS
60	3.2 ± 0.15	2.7 ± 0.15	NS	3.2 ± 0.3	3.5 ± 0.1	NS
120	4.2 ± 0.1	4.3 ± 0.1	NS	4.8 ± 0.3	5.0 ± 0.1	NS
180	5.2 ± 0.15	5.4 ± 0.3	NS	5.8 ± 0.3	5.9 ± 0.1	NS
240	6.1 ± 0.3	6.4 ± 0.3	NS	6.5 ± 0.3	6.6 ± 0.1	NS
	Saline i.v.	*Gla i.v. (1 mg)*		*Saline i.v.*	*Bov i.v. (1 mg)*	
30	1.9 ± 0.2	1.8 ± 0.3	NS	2.2 ± 0.2	2.2 ± 0.2	NS
60	2.5 ± 0.3	2.5 ± 0.4	NS	2.9 ± 0.3	2.8 ± 0.2	NS
120	3.6 ± 0.5	3.8 ± 0.6	NS	4.1 ± 0.3	3.9 ± 0.4	NS
180	4.9 ± 0.2	5.4 ± 0.5	NS	4.8 ± 0.3	5.1 ± 0.4	NS
240	5.7 ± 0.3	6.7 ± 0.5	NS	6.1 ± 0.3	6.2 ± 0.3	1.3
	Saline i.p.	*Glo i.p. (75 mg)*		*Saline i.v.*	*Pre-Sate i.v. (300 μg)*	
30	1.4 ± 0.1	1.3 ± 0.1	NS	1.9 ± 0.1	0.6 ± 0.1	.001
60	2.3 ± 0.2	2.1 ± 0.1	NS	2.6 ± 0.2	1.3 ± 0.2	.005
120	3.4 ± 0.2	3.7 ± 0.1	NS	3.7 ± 0.3	2.6 ± 0.2	.05
180	4.2 ± 0.2	4.7 ± 0.2	NS	5.2 ± 0.3	4.3 ± 0.3	NS
240	5.0 ± 0.1	5.6 ± 0.3	NS	6.3 ± 0.4	5.6 ± 0.2	NS