Effect of Gastrin on the *in Vivo* Incorporation of ^{14}C-Leucine into Protein of the Digestive Tract (34353)

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(Introduced by Morton I. Grossman)

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Johnson *et al.* (1) have recently shown that pentagastrin stimulated the incorporation of ^{14}C-leucine into protein of the digestive tract. In these experiments the *in vitro* incorporation of amino acids into protein of duodenal and gastric mucosal homogenates was increased at least 100% by treating the animals with pentagastrin 60 min before killing them. The effect of pentagastrin did not depend on blood flow or on the stimulation of acid secretion, for histamine had no effect on amino acid incorporation.

In order to determine whether gastrin has a physiological role in the regulation of protein synthesis within the mucosa of the digestive tract, as has been suggested (1), it is necessary to learn whether gastrin can increase the *in vivo* incorporation of amino acids into protein.

Methods. Male and female rats, weighing between 120 and 200 g, were fasted for 48 hr without restraint. The animals were randomly divided into control and experimental groups. All rats were injected intraperitoneally with uniformly labeled L-leucine-^{14}C (2×10^7 dpm/kg). At the same time animals from the experimental group received an intraperitoneal injection of either human synthetic gastrin (20 μg/kg) or ICI 50, 123 pentagastrin (250 μg/kg). These doses were found by Barrett (2) to produce 80–90% of the maximal acid secretory response when given to rats as single intravenous injections. Rats in the control group received saline injections of corresponding volumes.

Animals were killed 2 hr after injection. Their stomachs and proximal duodenums were quickly excised and placed in ice-cold saline. Samples of oxyntic, antral, and duodenal mucosa were obtained by scraping the tissue with a scalpel. Muscle from the oxyntic area of the stomach was obtained after the mucosa had been removed. Whole-thickness samples were taken from the squamous area of the stomach. Liver and skeletal muscle (thigh) samples were minced in cold saline.

Samples were homogenized in a final concentration of 5% (w/v) trichloroacetic acid (TCA) to precipitate protein (3); centrifuged and the precipitate was washed with cold TCA (5%); extracted with 5% TCA for 20 min at 90°, and washed again with cold 5% TCA (4). Two final extractions with ethanol:ether (3:1, v/v) were performed. The precipitate was suspended in 2 ml of H_2O and aliquots were taken for weighing and for counting. One aliquot was digested for 30 min in NCS (Nuclear, Chicago) and added to a counting vial. Radioactivity was determined in a Packard liquid scintillation counter. Counting efficiency did not vary significantly from 71% and results are expressed as counts per minute per milligram of dry weight of protein. The results were not corrected for quenching which was found to be negligible with this procedure.

Results. The results are shown in Table I. Both gastrin and pentagastrin stimulated the incorporation of radioactive leucine into protein of the gastrointestinal tract. No effect was seen on skeletal muscle or liver. The incorporation with gastrin ranged from 11.3 to 31.7% higher than the corresponding control values. Although the increase with gastrin was not great enough to be significant for all tissues tested, the results indicate that

TABLE I. Incorporation of ^{14}C-Leucine into Various Tissues of Rats Killed 2 hr after Injection with ^{14}C-Leucine and Gastrin or Saline.[a]

Tissue	^{14}C-Leucine (cpm/mg of dry wt)				% Stimulation
	n	Control	*n*	Gastrin	
Oxyntic mucosa	12	375.8 ± 20.2	12	442.9 ± 13.8	17.8[b]
Antral mucosa	12	230.0 ± 30.0	12	256.0 ± 24.9	11.3
Oxyntic muscle	12	137.8 ± 8.6	11	169.5 ± 15.0	23.0
				Pentapeptide	
Oxyntic mucosa	11	375.4 ± 30.6	11	427.3 ± 35.0	13.8
Duodenal mucosa	11	374.2 ± 33.8	11	474.6 ± 45.7	26.8[b]
Squamous stomach	11	92.9 ± 8.6	10	122.4 ± 11.3	31.7[b]
Liver	8	243.5 ± 2.9	8	239.4 ± 19.6	—1.7
Skeletal muscle	8	35.4 ± 8.7	8	29.0 ± 5.8	—22.0

[a] Means and standard errors of the means.
[b] $p < 0.05$.

gastrin causes a generalized increase in protein synthesis in the upper digestive tract.

The rates of incorporation were highest for duodenal and oxyntic mucosa. Samples of gastric muscle and gastric squamous area showed the lowest levels of protein synthesis. The reproducibility of this method of determining amino acid incorporation is shown by the near identity of the two separate control groups of oxyntic mucosa.

Discussion. In a recent symposium on gastric secretion Crean reported that moderate duodenal stenosis in the rat caused gastric mucosal hyperplasia (5). During the discussion of his paper he suggested that this effect might be mediated through a chronic overproduction of gastrin. There is other circumstantial evidence from clinical work that antrectomy, and presumably absence of gastrin, may lead to gastric atrophy (6).

Comparison of biopsy specimens from patients who have had either vagotomy or partial gastrectomy (antrectomy) for treatment of ulcer disease indicates that gastrin may be a trophic hormone for the gastric mucosa. Lees and Grandjean (7) performed biopsies on 33 "healthy" postgastrectomy patients. They classified only one of these as normal and 22 had from moderate to complete gastric mucosal atrophy. On the other hand, Melrose *et al.* (8) performed biopsies on 41 patients 1–10 years following vagotomy and found no instances of gastric atrophy. Both of these procedures reduce gastric secretion, but retention of the antrum apparently helps prevent mucosal atrophy.

In patients having Zollinger-Ellison syndrome there is a chronic overproduction of gastrin by tumor tissue (9). Mucosal hyperplasia and an increased parietal cell mass are characteristic of this disease (10). This is further evidence that gastrin may have a growth stimulating effect on the gastric mucosa.

Previous experiments have shown that pentagastrin doubles the *in vitro* rate of synthesis of protein in the mucosa of the oxyntic gland area of the stomach and trebles the rate of amino acid incorporation into protein of duodenal mucosal homogenates (1). It was also shown that this effect did not depend on blood flow or the secretion of hydrogen ion, for histamine in doses calculated to produce the same level of gastric secretion as the doses of pentagastrin employed had no effect on the *in vitro* incorporation of ^{14}C-leucine into protein (1).

The results of the current study are important in assessing the possible physiological role of gastrin in stimulating protein synthesis. These experiments show that gastrin increases the incorporation of amino acids into protein of the digestive tract, while not stimulating incorporation in liver and causing a decreased incorporation in skeletal muscle. These results from the *in vivo* system are qualitatively identical to those seen when in-

corporation was measured *in vitro*. The *in vivo* system is much less sensitive than the *in vitro* due to the dilution of isotope in the whole body, and the decreased chance for even distribution of isotope throughout the system. Nevertheless, significant stimulation was found in both gastric and duodenal mucosa.

The *in vitro* studies referred to were done with the synthetic pentapeptide containing the C-terminal tetrapeptide amide of gastrin (1). Therefore the current work demonstrates that there is essentially no difference between pentagastrin and the whole gastrin molecule in the ability to stimulate protein synthesis.

We interpret these results to mean that gastrin can stimulate amino acid incorporation in the live animal as well as in the *in vitro* systems, and infer that this is further evidence that gastrin may be a growth-stimulating hormone for parts of the digestive tract.

Summary. Rats were injected with ^{14}C-leucine and either gastrin or saline and killed 2 hr later. Protein was precipitated from various tissues and the incorporation of the radioactive amino acid determined as an index of protein synthesis. Gastrin produced an 11–32% stimulation of protein synthesis in tissues from the duodenum and stomach but had no effect on liver, and decreased the incorporation in skeletal muscle. These effects are qualitatively identical to those reported previously from an *in vitro* system. We conclude that this evidence further supports the suggestion that gastrin may be a trophic hormone for parts of the digestive tract.

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