

## INHIBITORS OF HISTAMINE CATABOLISM AND THE ACTION OF GASTRIN IN THE RAT

BY

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A method is described for the partial purification of hog's antral gastrin by gel filtration and ion-exchange chromatography. Gastrin was assayed by its effect on the pH of the effluent fluid from the perfused lumen of the stomach of the anaesthetized rat. The latency of the response to gastrin given intravenously was shorter than the latency to a similar dose of histamine. The response to gastrin injected into the arterial circulation of the stomach appeared sooner than the response to gastrin injected intravenously. Iproniazid and aminoguanidine potentiated responses to gastrin. Incubation *in vitro* with monamine oxidase or diamine oxidase did not inactivate gastrin. Chlorpromazine and bromolysergic acid diethylamide, in doses which enhance the effects of histamine on acid gastric secretion, did not affect responses to gastrin.

It has been shown, in dogs with Heidenhain pouches, that the intravenous injection of diamine oxidase inhibits gastric acid secretion induced by feeding (Peter, Walder, Sosin, Madsen & Wangenstein, 1962). It is therefore possible that the enzyme acts in some way to affect the action of gastrin. Injection of gastrin (Smith, 1954) and feeding (Schayer & Ivy, 1958) release histamine from the stomach, and the observations of Peter *et al.* (1962) could be explained by supposing that the injected enzyme increases the oxidation of histamine presumed to be released in the gastric mucosa. At least two assumptions are implicit in this account. Firstly, that increased gastric acid secretion induced by gastrin is mediated by the release of histamine and, secondly, that gastrin is not inactivated by diamine oxidase. The object of the present work was to test these assumptions. The first assumption was tested by studying the effect on the response to gastrin, of drugs which inhibit enzymes involved in the catabolism of histamine. Thus, diamine oxidase was inhibited by aminoguanidine, monamine oxidase by iproniazid, and imidazole-*N*-methyl transferase by chlorpromazine and bromolysergic acid diethylamide (Brown, Axelrod & Tomchick, 1959). All these drugs have been shown to enhance gastric secretion induced by histamine in rats (Ghosh & Schild, 1958; Amure & Ginsburg, 1964a). The second assumption was tested in experiments in which gastrin was incubated with diamine oxidase *in vitro*. Gastrin used in these experiments was prepared from porcine antra and was partially purified by the method described.

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In most previous investigations, the cat or the dog has been used for assaying gastrin activity. Here, activity has been estimated by the method of Ghosh & Schild (1958), in which acid secretion from the stomach of the anaesthetized rat is recorded continuously. Some characteristics of the response of the rat stomach to hog gastrin given intravenously, intra-arterially and intraportally are also described. In a paper published while this work was in progress, Lai (1963) has described a bioassay of gastrin in rats.

A preliminary account of this work has been published (Amure & Ginsburg, 1964b).

#### METHODS

*Perfusion of rat stomach in situ.* Male rats of albino and hooded strains weighing 150 to 300 g were used. The animals were anaesthetized with urethane (0.6 ml./100 g of a 25%, w/v, solution, intramuscularly) and the stomach was prepared for perfusion as described by Ghosh & Schild (1958). The perfusion fluid was an 0.001–0.00025 N-sodium hydroxide solution.

*Injection into the arterial blood supply of the stomach.* In rats anaesthetized with urethane, a fine polyethylene cannula was placed in the superior mesenteric artery. The aorta was tied immediately below the superior mesenteric artery and loops of thread, previously placed around the mesentery of the jejunum and ileum, were closed and the ileum and jejunum were excised. The hepatic artery was ligated and the spleen removed after ligation of the splenic artery. The blood to the stomach, duodenum and pancreas was supplied by the gastroduodenal, left gastric, right gastro-epiploic, superior and inferior pancreaticoduodenal arteries so that the injections, which were given slowly into the cannulated superior mesenteric artery, were driven through these vessels by the blood pressure. Animals tolerated the operation moderately well and experiments of 5 to 6 hr duration were possible.

*Intraportal injection.* In rats anaesthetized with urethane, the superior mesenteric vein was identified and isolated, taking care to preserve other tributaries of the portal vein. Loops were placed in preparation for the mass ligation of the intestinal tract (excluding the duodenum and the rectum). The superior mesenteric vein was cannulated and, as quickly as possible, the loops on the intestines and rectum were closed. The remaining sources of blood for the portal inflow were the spleen (lienal vein), the stomach (pyloric and gastric veins) and the pancreas and duodenum (superior and inferior pancreaticoduodenal veins).

*Preparation of gastrin.* Starting material was prepared by the method of Blair, Harper, Lake, Reed & Scratcherd (1961b); each batch was prepared from 500 g of hog antral mucosa. Further purification of this extract is described in Results.

*Protein and free amino-nitrogen estimations.* The Folin-Ciocalteu method was used for protein estimation following the procedure of Lowry, Rosebrough, Farr & Randall (1951); crystalline bovine albumin protein standard solution (Armour Laboratories) was used as standard. Free amino-nitrogen was estimated using ninhydrin-ascorbic acid reagent (D. K. Lewis & A. D. Sims, personal communication).

*Estimation of histamine.* The photofluorimetric method of Shore, Burkhalter & Cohn (1959) was used. Fluorescence was measured on a direct reading fluorimeter (Model 27A, Electronic Instruments Ltd., Richmond, Surrey) at an excitation wavelength of 3,655Å.

*Materials.* These were chlorpromazine (May & Baker); bromolysergic acid diethylamide (Sandoz Products); aminoguanidine carbonate (L. Light & Co.); iproniazid (Roche Products); hog liver monamine oxidase (Mann Research Laboratories); porcine kidney diamine oxidase (Calbiochem.).

#### RESULTS

##### *Assay of gastrin*

The assay of gastrin depended on changes of pH in the effluent fluid from the lumen of the perfused rat's stomach after intravenous injection of extracts containing

gastrin. The assays were used to "follow" the gastrin during purification. A "laboratory unit" was defined as the gastrin activity of 1 mg of the first crude extract prepared from hogs' antral mucosa by the method of Blair *et al.* (1961b). The effects shown in Fig. 1 were obtained in the same animal with 0.5, 1.0 and 1.5 units of gastrin and illustrate the consistency of the responses in an experiment lasting several hours. The response metameter chosen was the maximum fall in pH after the injection and in Fig. 2 response metameters are plotted against log dose of gastrin. The method is restricted to a rather narrow range of doses; the response is apparently maximal when the pH falls to a value about 3.5 because the effect on pH of further neutralization of alkali in the perfusing solution decreases as the pH is lowered.

Owing to the duration of the effect an interval of at least 40 min was required between doses. So as to be able to use a preparation for the assay of gastrin in more than one unknown sample, it was necessary, after the dose range for the unknown sample giving graded responses had been established, to limit the assays to a single

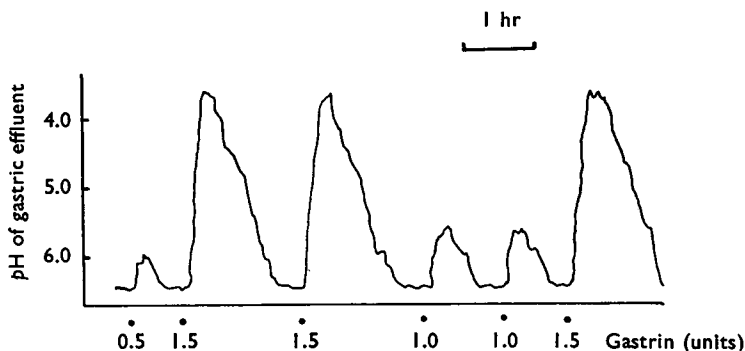


Fig. 1. Effects of repeated and varying doses of gastrin on the gastric effluent pH from the perfused stomach of an anaesthetized rat.

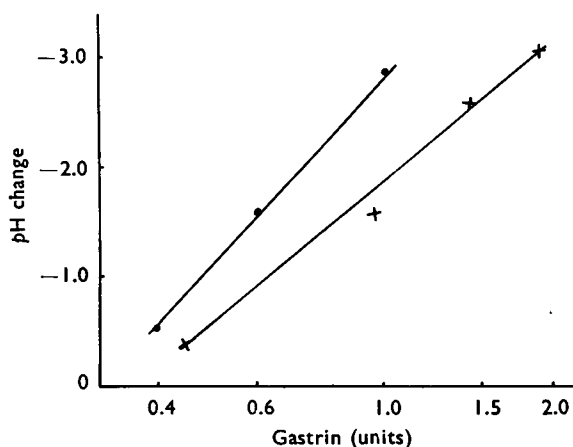


Fig. 2. Relationship between maximum fall in gastric effluent pH and dose of gastrin (log scale).

group of four doses consisting of a high dose and a low dose of the standard and the unknown.

#### Partial purification of gastrin

A suspension of the powder obtained by extraction of hogs' antral mucosa was stirred at 50° C in 0.15 M-sodium chloride solution for 1 hr and the water-soluble fraction was separated by centrifugation. The residue was extracted twice with 0.15 M-sodium chloride solution and the supernatant fluids were combined. About 50% (by weight) of the crude powder dissolved. Attempts to purify the gastrin were investigated on small samples taken from this extract; at each stage of purification gastrin activity, protein (Folin-Ciocalteu reaction) and free amino-nitrogen (ninhydrin) were estimated. The results are summarized in Table 1. 100 units

TABLE 1  
PARTIAL PURIFICATION OF HOG GASTRIN

Step	Fraction	Gastrin (units)	Protein (mg)	Free amino-nitrogen (mg)
1	Aqueous extract of crude powder	340	50	1.0
2	Sephadex G.25 gastrin-rich eluates	300	25.3	0.5
3	pH 3.2 precipitate	264	9.0	—
	supernatant fluid	85	14.6	—
4	Carboxymethylcellulose, pH 5.0 eluate	270	3.0	0.052

of the water-soluble fraction were placed on a Sephadex G-25 column and eluted with 0.15 M-sodium chloride solution. The results (Fig. 3) showed that the gastrin activity was eluted between the peak for protein and the peak for amino acids and small peptides. When a larger sample (340 units) was placed on the column, the gastrin-rich eluates contained 90% of the gastrin and less than 50% of the protein and free amino-nitrogen. The eluates containing gastrin were treated with glacial acetic acid added dropwise, until the pH reached 3.2. A precipitate formed and was separated by centrifugation. The precipitate was taken up in water and dissolved by addition of 1.0 N-sodium hydroxide solution to pH 7.0. 80% of the gastrin

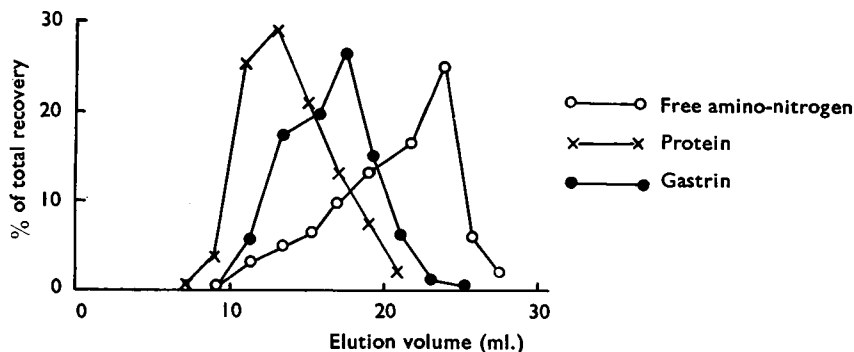


Fig. 3. Gel-filtration of aqueous extract of crude gastrin powder on Sephadex G.25 (30 × 1.2 cm); 100 units of gastrin in 0.15 M-sodium chloride solution were eluted with saline of the same concentration.

activity was recovered from the precipitate formed at  $pH$  3.2 which contained only 36% of the protein. The precipitate fraction was dissolved in pyridine acetate buffer  $pH$  5.0 (0.05 M-acetic acid/0.04 M-pyridine) and placed on a column of carboxy-methyl cellulose equilibrated with the same buffer at  $pH$  5.0. The column was eluted successively with pyridine acetate buffer at  $pH$  5.0 (0.05 M-acetic acid/0.04 M-pyridine),  $pH$  5.5 (0.05 M-acetic acid/0.08 M-pyridine) and  $pH$  6.0 (0.05 M-acetic acid/0.2 M-pyridine). All the gastrin activity was found in the  $pH$  5.0 eluate and more than 60% of the protein remained on the column. The final product contained 80% of the gastrin activity of the original crude extract with only 6% of the protein and 5% of the free amino-nitrogen.

#### *Time course of the response to gastrin*

In earlier experiments (Amure & Ginsburg, 1964a) we confirmed the observation of Ghosh & Schild (1958) that, after intravenous injection of histamine into anaesthetized rats, a latent period of about 6 min elapses before the  $pH$  of the effluent fluid from the stomach starts to fall. From the experiments with gastrin it was immediately obvious that the latent period was much shorter than with histamine; in all experiments the response to intravenous gastrin started within 2.5 to 3.5 min of injection. In three rats, each of which received more than one injection of both gastrin and histamine which produced responses of comparable size, the time (mean and standard deviation) to onset of the histamine response was  $6.2 \pm 0.2$  min (seven observations) and that for gastrin was  $3.2 \pm 0.15$  min (ten observations).

The part of the latent period due to the time taken for the perfusate to travel from the acid secreting region of the stomach to the glass electrode of the recording system was estimated at 35 to 45 sec. In rats given various doses of gastrin or histamine there was no obvious relationship between the size of the effect and its latency.

#### *Intra-arterial injection of gastrin*

Fig. 4,a shows effects of gastrin given by injection into the arterial supply of the stomach, duodenum and pancreas. The fall in the  $pH$  of the gastric effluent fluid

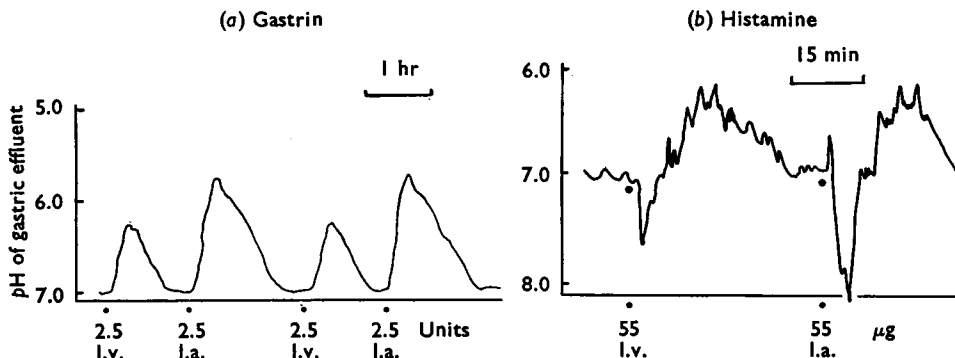


Fig. 4. (a) Effects of gastrin given intravenously (I.v.) and intra-arterially (I.a.) on the  $pH$  of gastric effluent fluid. (b) Effects of histamine given intravenously and intra-arterially.

was greater than that obtained when the same dose of gastrin was given intravenously. The latent periods of the responses to intravenous and intra-arterial gastrin were strikingly different. After intra-arterial gastrin the response started within 1 min of injection compared with a latent period of approximately 3 min when gastrin was given intravenously. Since, as noted above, the time taken for the perfusate to clear the dead space between the stomach and the electrode is 35 to 45 sec, it would appear that the response to intra-arterial gastrin occurs almost immediately. Fig. 4,*b* shows an experiment in which histamine was given intra-arterially and intravenously to a rat. The response of this animal to histamine given intravenously was atypical in that there was a slight increase in effluent pH before the increase in acid secretion occurred. When the same dose of histamine was given intra-arterially the preliminary phase (increase in pH) was more pronounced and the subsequent acid secretion was not greater than after intravenous injection.

#### *Intra-portal injection of gastrin*

Effects on acid gastric secretion of gastrin given intravenously (external jugular vein) and intraportally (superior mesenteric vein) were compared in rats prepared for injection by both routes. All injections were given slowly over a period of

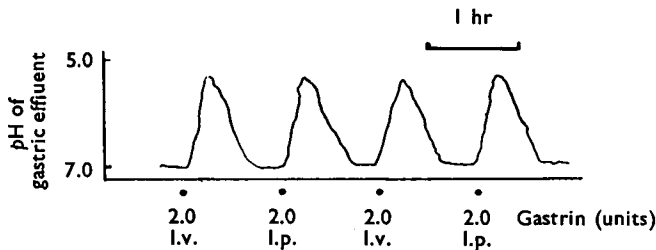


Fig. 5. Effects of gastrin given intravenously (I.v.) and intraportally (I.p.) on the pH of gastric effluent fluid.

50 sec. Fig. 5 shows the results of a typical experiment. The size and time course of the response to gastrin given intraportally is not perceptibly different from that to the same dose given intravenously.

#### *Effect of gastrin after treatment with drugs which enhance the effect of histamine*

The effects of aminoguanidine and iproniazid on responses to gastrin are shown in Fig. 6,*a* and *b*. Gastrin was given in a dose which gave a consistent but small increase in gastric acid secretion (maximum fall in effluent pH < 1.0 pH unit) before treatment with aminoguanidine or iproniazid. When the same dose of gastrin was given 3 min after the injection of 3 mg of aminoguanidine or 3 mg of iproniazid the effect on gastric acid secretion was increased and, in the experiment with iproniazid, even 1 hr later the response was greater than before injection of the monamine oxidase inhibitor.

In preliminary experiments it was found that treatment with chlorpromazine and bromolysergic acid diethylamide, in doses which in previous experiments

enhanced the effect of histamine, did not affect the response to gastrin. In the experiments shown in Fig. 7, *a* and *b* both gastrin and histamine were given to the same animal before and after chlorpromazine or bromolysergic acid diethylamide. While both drugs enhanced responses to histamine, the effects of gastrin were unaltered.

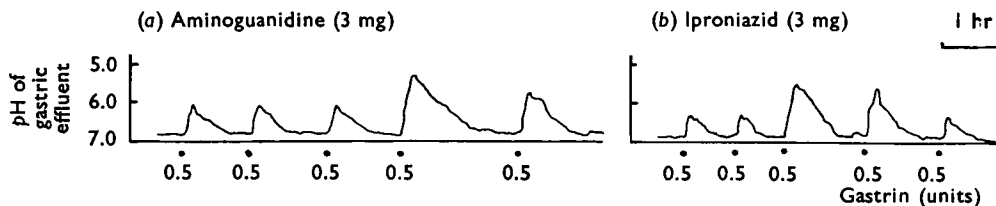


Fig. 6. Effects of gastrin before and after treatment with (a) aminoguanidine given 3 min before the fourth dose of gastrin, and (b) iproniazid given 3 min before the third dose of gastrin, on the pH of gastric effluent fluid.

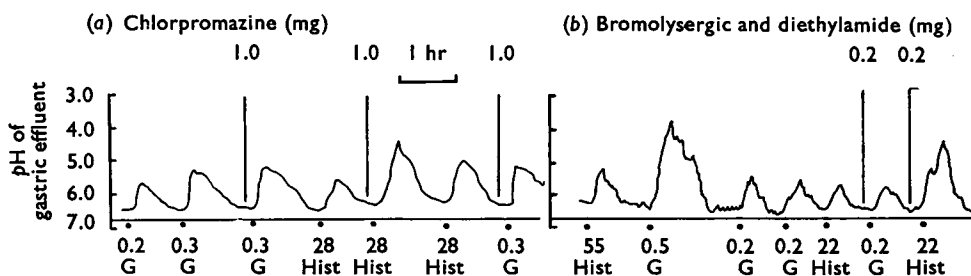


Fig. 7. Effects of gastrin (G; in units) and histamine (Hist; in  $\mu\text{g}$ ) on pH of gastric effluent fluid before and after treatment with (a) chlorpromazine, and (b) bromolysergic acid diethylamide.

#### *Incubation of gastrin with diamine oxidase and monamine oxidase*

From present knowledge of the structure of gastrin (Gregory & Tracy, 1963) it seems unlikely that it could be a substrate for diamine oxidase or monamine oxidase. However, since the effect of gastrin is enhanced by aminoguanidine and iproniazid and in view of the observations of Peter *et al.* (1962) it was necessary to test the effect of the enzymes on gastrin activity *in vitro*.

Gastrin (12 units) was incubated at 37° C for 24 hr in 0.5 M-sodium phosphate buffer at pH 7.2 in the presence of 0.5, 0.25 and 0.05 mg of diamine oxidase, following the conditions specified by Kapellar-Adler & Renwick (1956). The reaction was stopped by heating for 5 min at 100° C and, after centrifugation, gastrin activity was assayed in the supernatant fluid. Recovery of gastrin activity was 84 to 100% of the gastrin added to the incubate. When 10  $\mu\text{g}$  of histamine were incubated under the same conditions, less than 1% of the histamine remained. Gastrin (20 units) was incubated for 30 min at 37° C in 0.08 M-phosphate buffer, pH 7.4, in the presence of 0.5, 0.25 and 0.05 mg of monamine oxidase, following the conditions described by Lovenberg, Levine & Sjoersdma (1961). The reaction was stopped and gastrin assayed as described above. Of the gastrin activity, 80 to 85% was recovered.

## DISCUSSION

In our experiments the actions of gastrin and histamine on acid secretion in the rat stomach have been shown to differ in three respects. First, the latency of the response to intravenous gastrin was shorter than the latency of the response to histamine given intravenously; secondly, when gastrin was given intra-arterially the fall in the pH of the gastric effluent fluid was greater than after intravenous injection of the same dose, whereas the effect of intra-arterial histamine was not greater than that of intravenously administered histamine; thirdly, the effect of histamine was enhanced by previous treatment with chlorpromazine and bromolysergic acid diethylamide, whereas the response to gastrin was unaltered.

The shorter latency of the response to gastrin has not been reported previously; Ghosh & Schild (1958) found a latent period of 6 to 10 min with both histamine and choline esters in anaesthetized rats, but their conclusion that the latency is characteristic of the secretory system is not supported by the more rapid onset of the response to gastrin given intravenously and the almost immediate effect of intra-arterial gastrin. The different intervals elapsing between injection and response to histamine and to gastrin might be explained by supposing that the gastrin passed rapidly to sites in the stomach where it released histamine which then acted to stimulate acid secretion. Histamine released in this way would be expected to act with greater speed than histamine injected into the circulation.

Scattered observations on the effects of gastrin and histamine given intravenously and into the arterial supply to the stomach in cats agree with the present findings in rats. Thompson & Vane (1953) found that histamine injected intra-arterially into the cat did not have a greater effect on acid secretion than did intravenous injection; Smith (1954) found that the effect of gastrin was greater after intra-arterial injection. However, as Hollander & Shapira (1963) have pointed out recently, the gastric response to histamine in rats is complicated by "non-parietal" effects and by effects on the gastric circulation which would not be expected to occur if histamine was liberated by gastrin in the region of the parietal cells.

Westling (1958) has shown that methylation is an important pathway of histamine metabolism in male rats. Nevertheless, chlorpromazine and bromolysergic acid diethylamide, which are inhibitors of imidazole-*N*-methyl transferase (Brown, Axelrod & Tomchick, 1959) and which enhance the effects of histamine on gastric secretion in the rat (Amure & Ginsburg, 1964a), do not affect responses to gastrin, whereas inhibitors of diamine oxidase and monamine oxidase potentiate effects of both gastrin and histamine. The explanation may be that, since the rat's stomach contains diamine oxidase (Kobayashi & Ivy, 1959) but lacks imidazole-*N*-methyl transferase (Brown *et al.*, 1959), methylation in the ring may not determine the fate of histamine released in the rat stomach.

All the present observations are consistent with the view that the action of gastrin is mediated through histamine release, and that, in the main, the effect is due to histamine liberated in the gastric mucosa. This conclusion agrees with observations of Schayer & Ivy (1958) that feeding reduces the labelled histamine content in the stomach of rats previously injected with [<sup>14</sup>C]-histidine, and can be reconciled with



those of Blair, Dutt, Harper & Lake (1961a) who showed that, in cats, stimulation of gastric acid secretion with gastrin was not associated with an increase in the histamine content of arterial plasma.

The experiments in which gastrin was given intraportally were prompted by the observation of Gregory (1958) that, after portal vein ligation, gastric secretory responses to feeding were much increased in dogs with denervated stomach pouches. Hypersecretion of acid in the stomach had been reported earlier by Lebedinskaja (1932) and by Gerez & Weiss (1937) after creation of Eck fistulae in dogs with Pavlov pouches; Gregory's experiments established that this effect was not due to nervous influences or to the liver atrophy which occurs in dogs with Eck fistulae. Since all these workers suggested that impaired destruction of a hormonal gastric secretagogue, normally inactivated in the liver, might be responsible for their findings, it seemed appropriate to compare the effects of gastrin given intravenously and intraportally.

The finding that gastrin injected into the portal vein gave as great a secretory response as did injection into a jugular vein confirms similar findings by Gillespie & Grossman (1962) in dogs. Gillespie & Grossman (1962) interpreted this result as showing that gastrin is not normally extracted from the blood during circulation through the liver and that, therefore, the effects of porto-caval anastomosis (and portal vein ligation) are due to increased sensitivity of parietal cells. These experiments show that Gregory's (1958) suggestion that "the gastric secretory stimulant, whatever its nature, does not survive passage through the liver" cannot apply to gastrin, without qualification. If, say, only 10% of the gastrin in blood was extracted during each circulation through the liver it is unlikely that differences in responses to intravenous and intraportal gastrin would be detected. However, provided that there are no important extrahepatic sites for inactivation of gastrin, the extinction of a quite low rate of extraction of gastrin in the hepatic circulation could be responsible for enhanced secretory responses after portal vein ligation.

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