Gastrointestinal Hormones

International Symposion at Erlangen, August 1971

Edited by

Ludwig Demling

100 Figures, 15 Tables

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Contents

Release of Gastrin in Relation to Antral Acidity and Absorption of Chemical Stimulants *

S. ANDERSSON and C.E. ELWIN

Our knowledge of the intimate mechanism which controls the release of antral gastrin in response to various topically applied agents such as amino acids and alcohols is very limited. It is, however, known that there exist some structure-activity relationships as far as amino acids and alcohols are concerned (Elwin and Uvnäs 1966, Elwin 1969 a). For example, n-propanol is a strong gastrin releaser but isopropanol is not; and, α-alanine is a poor gastrin releaser, whereas β-alanine is very effective. Furthermore, we know that the degree of acidity of the antral content is of major importance; gastrin release is facilitated at neutral pH-values whereas release is inhibited below pH 2.

We have studied in dogs with isolated antral and fundic pouches the release of gastrin in response to some chemical stimuli at various antral pHs and correlated their gastrin releasing efficacy with the degree of absorption.

Methods

Adult mongrel dogs weighing between 10 and 19 kg were provided with innervated fundic pouches (Pavlov pouches) and innervated antral pouches. Gastric secretory outputs were measured from the Pavlov pouches in response to various gastrin releasing agents. Stimuli for gastrin release were ethanol, n-propanol and choline chloride. Solutions of these agents were either perfused (100 ml/hr) through or kept (30-40 ml) in the antral pouch for various periods of time (1-3 hours). All test agents were administered in buffer solutions (Mac Ilvaine's buffer) of different pHs.

Ethanol and n-propanol concentrations in the antral perfusate and in blood were determined by gas chromatography (Bonnichsen et al. 1967). Free choline in plasma was determined by the method described by Smith, 1957. In experiments in which the disappearance of alcohol from the antral perfusate was measured, polyethylene glycol (PEG 3000) was added to the test solutions (2 mg/ml).

Results

Introduction of 10% ethanol into the antral pouch for three hours gave rise to a secretory response from the Pavlov pouch of about one and one-half hours duration (Fig. 1). During the latter part of the instillation period the response disappeared. Renewal of the alcohol solution produced a second secretory response of the same magnitude and duration as the first one.

Determination of ethanol in both antral fluid and in peripheral venous blood revealed a relatively rapid transfer of ethanol from the antral pouch to blood during the first hour (Tab. 1 and Fig. 2). It can be seen (Tab. 1) that with 15% ethanol about

* The study has been supported by the Swedish Medical Research Council (B71-14X-88-07C) and by Karolinska institutet.

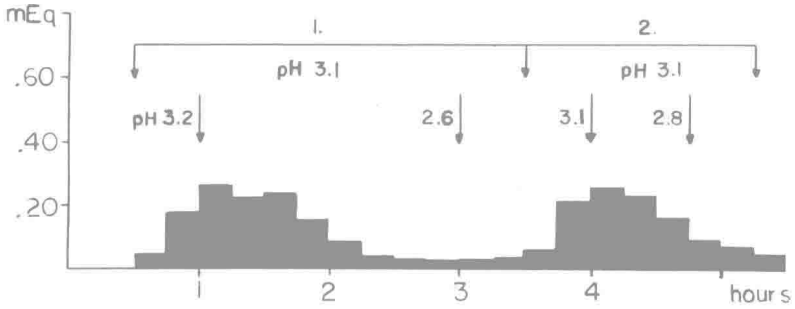

Fig. 1: Gastric acid output in response to antral stimulation with ethylalcohol. 1) Antral instillation for 3 hours of 30 ml of 10% ethanol at pH 3. 2) Reinstillation for 2 hours of 10% ethanol at pH 3.

Table 1: Disappearance of ethanol from the isolated antral pouch. Secretory output from the fundic pouch in one dog is shown together with the range of ethanol concentrations in the antrum and in peripheral venous blood after certain time intervals during instillation of 8% (4 experiments) and 15% (2 experiments) ethanol solutions at pH 7.

		8% (v/v) EtOH			15% (v/v) EtOH		
	Time	Acid output mEq/hr	Antrum EtOH conc (W/V)	Venous blood EtOH conc	Acid output mEq/hr	Antrum EtOH conc (W/V)	Venous blood EtOH conc
		mean	%	‰	mean	%	‰
	0' controls	0.09	6.60-6.74	0.00	0.04	11.7	0.00-0.00
							0.00-0.05
						3.70-5.45	0.00-0.08
						2.95-3.70	0.04-0.07
	60'	0.86	1.10-1.70	0.03-0.05	0.84	2.55-2.90	0.03-0.06
Instillation	120'	1.02	0.32-0.88	0.00-0.01	0.51	0.12-0.60	0.00-0.02
	180'	0.44	0.18-0.52	0.01-0.02	0.18	0.14	0.00-0.01
	240'	0.42					

two thirds of the alcohol had disappeared from the pouch within 30 minutes; there was no significant rise in the alcohol concentration in peripheral venous blood (Tab. 1 and Fig. 2). From Table 1 it can also be seen that there was a direct correlation between gastric acid output and the concentration of ethanol in the antral pouch.

In another series of experiments the disappearance of ethanol and n-propanol from the antral pouch at different pH-levels was studied (Fig. 3). This study showed that there were no major differences between absorption of the alcohols at the various pH-levels. Thus, the absorption of alcohols through the antral mucosa seems to be completely independent of pH. On the other hand, secretory responses of the fundic pouches to antral stimulation with ethanol are strongly inhibited at pH 1, possibly by a direct action of acid on the gastrin releasing cells (Andersson and Elwin, 1971). Secretory responses to ethanol at pHs 7 and 3 are identical.

Gastrin release following antral perfusion with graded doses of choline showed a pronounced pH-dependence. (Fig. 4). For example, secretory responses to antral

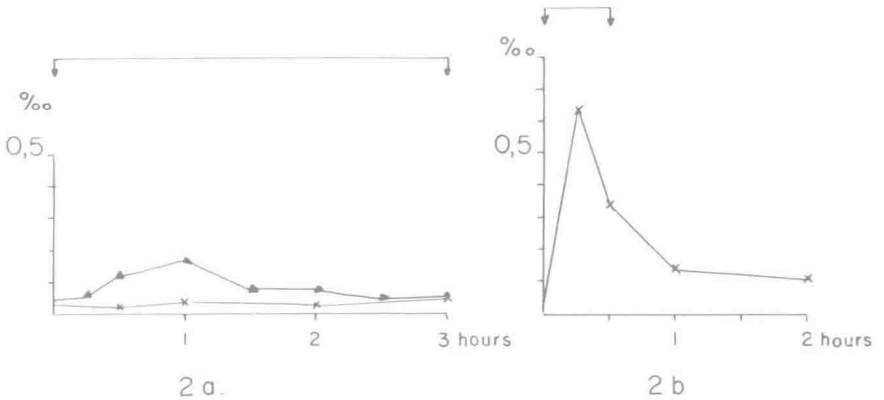

Fig. 2: Blood concentrations of ethanol (‰) during antrum application.
= period of instillation or perfusion. a. 3-hour antral instillation of 32% ethanol.
× = ethanol in peripheral venous blood △ = ethanol in portal blood
b. 30-minute antral perfusion with 32% ethanol in an acute experiment.
× = ethanol in portal blood

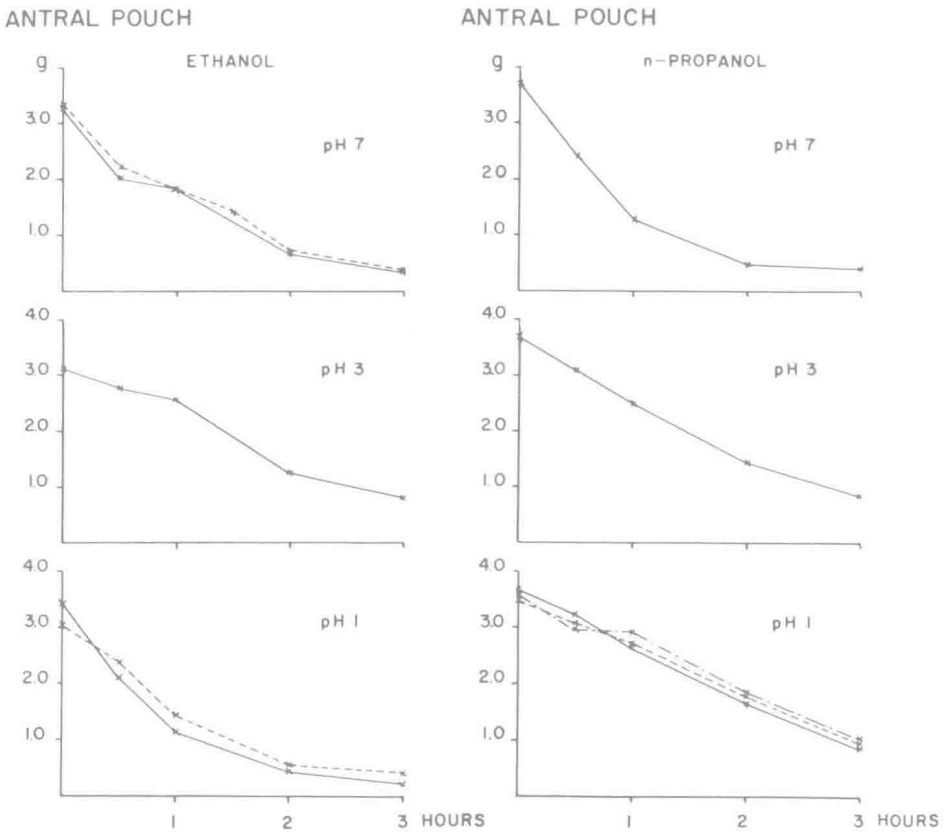

Fig. 3: Disappearance of ethanol and n-propanol from the antrum pouch at different pH-levels. Instillation for 3 hours of 30 ml of 16% solutions.

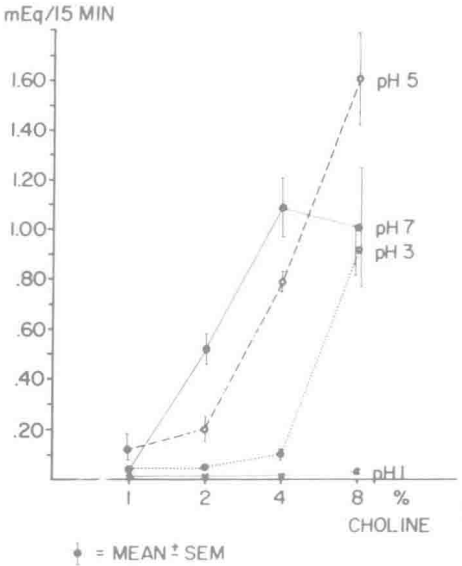

Fig. 4: Gastric acid output to antral stimulation with graded concentrations of choline at different pH-levels in three Pavlov pouch dogs. Each point is the mean acid output for the last two 15 min. periods at each dose.

perfusion with 2% choline were significantly lower at antral pH of 5 than when the antral pH was 7. With 8% choline at antral pH 7 the animals showed typical sings of general cholinergic excitation, indicating absorption of choline through the antral mucosa. No such sings were observed when the pH of the choline solution was 5 or less. Determination of choline in blood also showed a rise when the antrum was perfused with 8% choline at pH 7 (Fig. 5); with lower concentrations of choline, no difference from control values could be detected.

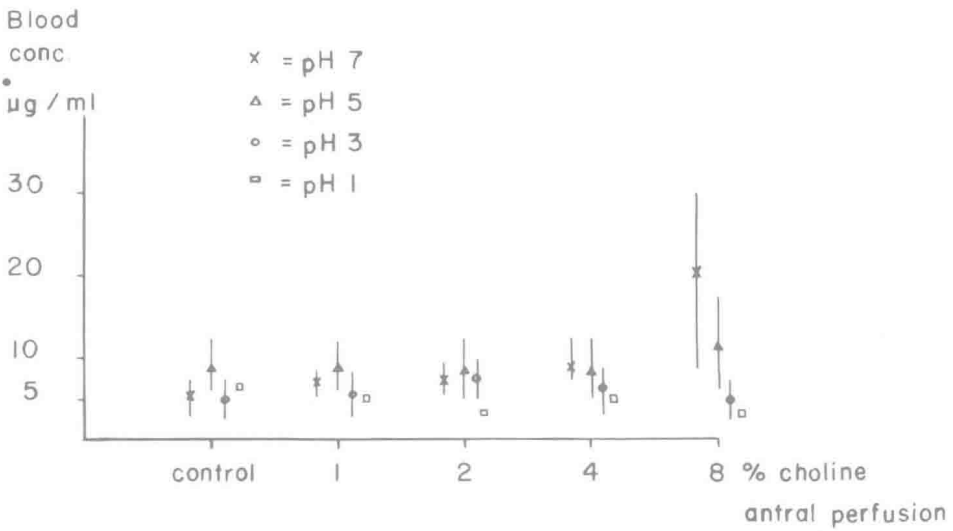

Fig. 5: Blood concentrations of choline during antral perfusion with graded doses of choline at different pH-levels. Symbols indicate mean blood concentration at the end of each 1.5-hour perfusion. Vertical bars denote the range.

Discussion

This study has demonstrated that potent gastrin releasing agents such as alcohols and choline are absorbed through the antrum mucosa. It has also been shown that the absorption of ethanol and of n-propanol occurs completely independently of the pH of the antral perfusate. On the other hand, the experiments with choline suggest a pH-dependent absorption, though significant differences between blood levels of choline at different antral pH-levels could only be detected when the highest concentration of choline was used (8%).

Thus, contrary to the suggestion by Woodward et al. (1957), it has been demonstrated that ethanol disappeared rapidly from a solution instilled into the isolated antral pouch. The disappearance of ethanol was not due to any metabolic breakdown, since no metabolites of ethanol could be detected in the instillate by gas chromatography. Proof of the absorption of ethanol from the isolated antral pouch was provided by an immediate rise in the ethanol concentration in portal blood.

During irrigation of antrum with ethanol the concentration of ethanol in peripheral venous blood was insignificant. The concentration of ethanol in blood necessary for direct stimulation of the parietal cells is not known. However, by intravenous infusion of graded doses of ethanol the threshold blood level of ethanol for stimulation of the parietal cells was found to be of the order of 0.2-0.3‰ (Elwin, to be published). Therefore, a direct stimulatory effect of ethanol on the parietal cells in the present experiments was unlikely (Tab. 1). Consequently, the secretory responses of the fundic pouches observed during antral irrigation with ethanol were obviously due to release of gastrin.

Gastrin release by ethanol is greatly inhibited below pH 2 (Elwin 1969 b, Andersson and Elwin 1971). On the other hand, the curves for the disappearance rate of ethanol from the antrum clearly demonstrated that high acidity did not interfere with the absorption of the alcohol. Thus, acid inhibition of gastrin release by ethanol cannot be explained by either mucosal damage by acid or by decreased absorption of the alcohol. Furthermore, since ethanol is a unionizable compound, the loss of gastrin releasing potency at low pH could not be due to chemical changes in the ethanol molecule.

The experiments in which gastrin release was stimulated with choline revealed a rather different pH-dependency compared with that of ethanol. Thus, a significant inhibition of gastrin release in response to choline could already be demonstrated at pH 5. Complete inhibition of gastrin release occurred when the solutions of choline had been acidified to pH 1. Therefore, we assume that two inhibitory mechanisms are involved in the acid induced inhibition of the gastrin release induced by choline. At very low pH-values (pH 1-2) acid may make the gastrin mechanism generally unresponsive to all gastrin releasing stimuli. The assumption is supported by the fact that gastrin release by local chemical stimuli, as well as vagal release of gastrin, is abolished at these pH-levels (Andersson and Olbe 1964). It is, however, unlikely that the marked inhibition of gastrin release by choline at pH 5 could be explained by this mechanism. It was observed that in each instance when the antrum was perfused with 8% choline at pH 7 the dogs exhibited pronounced signs of cholinergic excitation, indicating absorption of choline. The same symptoms have previously been observed during perfusion of canine isolated intestinal loops with choline (Rohse and Searle, 1955). As the pH of the 8% choline solution was decreased to pH 5 and then to pH 3 these symptoms disappeared completely. By measuring free choline in plasma, absorption of choline (8%) at pH 7 could be confirmed by a significant increase in the plasma level of choline. At pH 5 no significant increase in plasma choline above control levels could be detected. Evidently, choline can be

absorbed through the antral mucosa and, furthermore, our results indicate that the absorption is pH-dependent, decreasing as the pH falls. It was surprising to find that the absorption of choline was pH-dependent, since it is a strong base and is accordingly completely ionized at all pH-levels that we have studied. According to generally accepted views (Schanker et al. 1957; Levine 1959), completely ionized quaternary amines are poorly absorbed from the gastrointestinal tract and, furthermore, absorption should not be pH-dependent. Therefore, the mechanism of transport of choline across the antral mucous membrane is obscure. The fact that the reduced absorption of choline runs parallel to decreased gastrin release might be suggestive evidence of one mechanism for inhibition of gastrin release. Therefore, our hypothesis is that one prerequisite for an effective gastrin releasing agent is that it has to be absorbed through the antral mucosa. If absorption occurs, it would facilitate the transport of gastrin releasing agents to the gastrin cells and thereby gastrin release. Therefore, it becomes unnecessary to postulate the existence of a local reflex arc with receptors at the mucosal surface involved in the release of gastrin, and, in fact, there is no convincing evidence for such a reflex mechanism.

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Radioimmunoassay of Serum Gastrin in Patients with Peptic Ulceration Using Antiserum Against Pentagastrin

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Introduction

The advent of radioimmunoassay technique (1) has provided a sensitive and reproducible method for the determination of small quantities of glycoproteins, peptides and, more recently, steroids. It is important to realize, however, that radioimmunoassays have two omportant sources of error – (1) cross-reaction in the assay system by substances containing a similar antigenic sequence to the substance being measured, e.g. cross-reaction by "gut-glucagon" in assays for pancreatic glucagon, (2) and (b 2) dissociation of biological and immunoassayable activity (3). This latter situation is always a hazard when antiserum used for radioimmunoassay is produced against any antigenic sequence other than the site of biological activity. It can result in either fallaciously elevated or depressed (relative to biological potency) estimations if preferential metabolism or damage occurs in the biological or immunological sequence respectively. Since pancreozymin-cholecystokinin (PZ-CCK) has an identical C-terminal pentapeptide sequence to the biologically active site of heptadecapeptide gastrin (4) it follows that one or the other potential hazard will occur with any radioimmunoassay for gastrin. Thus an assay using antiserum specifically against the C-terminal sequence of gastrin will inevitably suffer lack of specificity due to cross-reaction by PZ-CCK. If cross-reactivity by PZ-CCK is avoided by using antiserum specific for the N-terminal sequence of heptadecapeptide gastrin bio-immunological dissociation may occur. Most radioimmunoassays (5, 6, 7) have avoided significant cross-reaction with PZ-CCK by accepting the latter hazard. In this paper the serum levels of gastrin observed with a radioimmunoassay using antiserum against the C-terminal fragment of gastrin are reported and the significance of cross-reaction by pancreozymin assessed.

Methods and Materials

Gastrin Assay

Serum gastrin determinations were made using a radioimmunoassay described in detail elsewhere (8). Briefly, Pentagastrin (Imperial Chemical Industries, U.K.) 40 mg was conjugated to 100 mg of rabbit serum albumin (Mann Res. Lab. N.Y.) using a water soluble carbodiimide (1-ethyl-3-(3-dimethyl-aminoprpyl) carbodiimide, (K and K Lab. Calif.) according to the method of Goodfriend et al (9). After dialysis the conjugate was lypophilized and 4 mg of the conjugate in 0.5 ml of saline was emulsified in an equal volume of Freund's Complete Adjuvant (Bioquest, Maryland, U.S.A.) and injected bimonthly IM into the hindquarter of five white Leghorn chickens. Serum was obtained by monthly bleedings from the wing-vein.

Synthetic human gastrin I (Imperial Chemical Industries, U.K.) was iodinated by the Chloramine T method of Hunter and Greenwood (10). Gel filtration (Sephadex GlO - Pharmacia, Sweden) was used to remove the unreacted ^{125} or ^{131} iodide. Using 5 μg

synthetic human gastrin I (hGl) and 2 mCi I^{125} (Amersham I.B.S. - 3) specific activities of 150 to 250 mCi/mg were recorded.

The assay procedure consisted of preincubating 0.5 ml of serum (or standard solutions of hGl in 3.75% gelatine) with 0.5 ml of appropriately diluted antiserum (1/500). All dilutions were made with 0.1 M NaCl, 0.08 M phosphate buffer pH 7.4 containing 0.5% W/V Bovine serum albumin (Armour, U.K.) and the reactants incubated in 20 ml glass scintillation vials. After two hours at 20°C, 10 pg hG1 ^{125}I in 4 ml of buffer were added to each vial. A further 14 hours incubation at 20°C was followed by the batchwise addition of 200 mg Amberlite CG 400 type II resin (Rohm and Haas, Phil. U.S.A.) in the hydroxide cycle according to the method of Lazarus and Young (11). After washing twice with distilled water, the resin (which bound the "free" ^{125}I hG1) was counted in a Nuclear Chicago Auto spectrometer and the count expressed as a percentage of resin uptake (or "free"/total).

Pancreozymin-cholecystokinin determinations were performed by the late J.D. Young. Sensitivity of this assay was teported to be 15 Crick, Harper, Raper μU/ml (12). The assay was specific for pancreozymin with no cross-reaction by gastrin or gastrin analogs.

Patients and Control Subjects

The normal control subjects (41) consisted of medical or paramedical personnel with no personal or family history of gastrointestinal disease.

The patiens with peptic ulceration had radiological evidence of an "active" duodenal (40) or radiological ± gastroscopic evidence of a benign lesser curve gastric ulcer (12). Sixteen patients were examined six weeks to five years after truncal vagotomy for duodenal ulceration. These patients were considered to have a "complete" or "incomplete" vagotomy according to Hollander's interpretation (13) of the gastric-acid response to insulin induced hypogliycemia (a "negative" Hollander response was considered to be synoymous with "complete vagotomy"). A further seven patients were examined after partial gastrectomy for duodenal ulceration. These were divided into two groups according to the presence (4) or absence (3) of recurrent ulceration.

Physiological Studies

1) Vagal stimulation was induced by either sham-feeding (3 normal subjects) or insulin-induced hypoglycemia (3 patients with duodenal and 2 patients with gastric ulceration). The gastric contents were continuously aspirated during the period of hypoglydemia.
2) A standard protein meal (2 "Oxo" cubes in 150 ml of water) was administered to 7 normal subjects, 12 patients with duodenal and 11 patients with gastric ulceration.
3) Hyperglycemia was induced by either oral ingestion of 50 G glucose (3 normal subjects) or intravenous infusion of glucose at 0.75 G/min. over 20 minutes (5 normal subjects and 7 patients with duodenal ulceration).
4) Atropine sulphate (25 μg/kg) was injected I,V, over one minute into two normal subjects and two patients with duodenal ulceration.
5) Antral acidification was induced in six patients with duodenal ulceration by stimulating parietial-cell secretion with betazole HCI ("Histalog"-Elli Lilly)

50-100 mg IMI. Antral pH was monitored by an intragastric pH electrode or by aspiration of small alliquots of gastric juice via an I.G. tube.

Results

Antiserum obtained from one chicken after five months of immunization bound 94% of ^{125}I hGl under conditions of antibody excess. Addition of unlabelled hGl inhibited antibody binding of ^{125}I hGl as did PZ-CCK, Pentagastrin and serially diluted serum from a patient with a Zollinger-Ellison tumor. Optimal dilution of antiserum (i.e. the dilution giving the largest F/T 5 ng – F/T 0.0 ng) was found to be 1/500. At this dilution 93% of ^{125}I hGl was antibody bound. Addition of 0.05 and 5.0 ng hGl/0.5 ml produced a decrease in ^{125}I hGl bound to 85% and 10% respectively. (Fig. 1). Sensitivity of the assay (defined as I.S.D. of R_0 from 0 hormone conc.) (14) was 9 pg/ml. Coefficient of variation for samples within and between assays was< 7%.

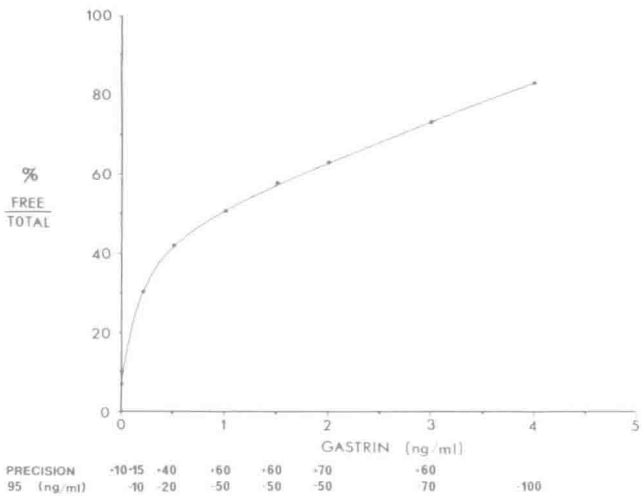

Fig. 1: A standard curve for radioimmunoassay of gastrin using antiserum against pentagastrin.

The dose-response inhibition curves for hGl, PZ-CCK and serially diluted serum were parallel. Pentagastrin, although used to produce the antibodies, gave a dose-response curve of "partial-reaction" when ^{125}I hGl was used as the tracer (Fig. 2). The parallel dose-response curves for hGl and PZ-CCK allowed calculation of relative inhibitory potencies of these hormones. Figure 3 demonstrates that 1000 C.H.R. μU PZ-CCK were equivalent to 50 pg/hGl. Thus the effect of known levels of added (or measured) pancreozymin in the assay system could be calculated and expressed ea "ph/equiv. of hGl".

Fasting serum levels of PZ-CCK in 46 control subjects were <15 C.H.R. μU/ml (i.e. <0.75 pg/equiv. hGl.) PZ-CCK levels after vagal stimulation did not exceed 90 μU/ml (4.5 pg/equiv. hGl.), while levels after ingestion of fatty or mixed meals reached 2,400 μU/ml. The latter response is illustrated, together with similtaneous