Pure Human Big Gastrin

IMMUNOCHEMICAL PROPERTIES, DISAPPEARANCE HALF TIME, AND ACID-STIMULATING ACTION IN DOGS

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ABSTRACT Biological properties of pure natural human "big gastrin" (designated G-34 because it contains 34 amino acid residues) were compared with those of pure natural heptadecapeptide gastrins (G-17) from human and porcine sources. Radioimmunoassay inhibition curves indicated that G-17 was nearly 1.5 times more potent than G-34 with the antibody used in this study. This difference was confirmed by demonstration of increased immunoreactivity generated when G-34 was converted to G-17 by trypsinization.

When infused intravenously into dogs with gastric fistulas and Heidenhain pouches in equimolar doses, G-34 produced slightly higher acid secretory responses than G-17. Responses to sulfated and nonsulfated forms were not significantly different, nor were responses to human and porcine G-17.

During infusion of equimolar doses, steady-state serum gastrin concentrations were more than fivefold higher with G-34 than with G-17. The difference in steady-state blood concentrations could be accounted for by a corresponding difference in removal rates. The half times of the G-34 preparations averaged 15.8 min and the half times of the G-17 preparations averaged 3.2 min. The calculated spaces of distribution for G-17 and G-34 were similar, about 25% of body weight.

When the increment in serum gastrin was plotted against acid secretory response it was found that nearly five times greater increments in molar concentrations of G-34 than of G-17 were required to produce the same rate of acid secretion. The potency of these two molecular forms of gastrin can be expressed in two different ways. Based on exogenous molar doses, the potencies of G-34 and G-17 were similar. However, based on molar increments in serum gastrin concentration, G-17 was approximately five times more potent than G-34. Hence, fractionation of these gastrin components may be important in estimation of the acid-stimulating action represented by total serum gastrin as measured by radioimmunoassay.

INTRODUCTION

Radioimmunoassay systems currently employed for measurement of gastrin all use heptadecapeptide gastrin (G-17)[1] as the standard, most commonly nonsulfated human gastrin I (HG-17-I), porcine gastrin I (PG-17-I), or sulfated porcine gastrin II (PG-17-II) (1–3). Yalow and Berson recently reported that the principal circulating form of gastrin in hypergastrinemic humans was a larger molecule which they named "big gastrin" (4). This observation has been extended by them (5, 6) and has been confirmed by others (7, 8).

Gregory and Tracy extracted human big gastrin from gastrin-secreting tumors of the Zollinger-Ellison variety and have showed that it contains 34 amino acid residues (9). Both sulfated and nonsulfated forms were identified. Throughout the remainder of this paper HG-34-I and HG-34-II will be used to designate the nonsulfated and sulfated forms of human big gastrin.

The purpose of the present investigation was to compare G-34 and G-17 preparations in regard to acid stimulation, rates of elimination from the circulation, and

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[1] *Abbreviations used in this paper:* G-17, little gastrin; G-34, big gastrin; HG-17-I, nonsulfated human little gastrin; HG-34-I, nonsulfated human big gastrin; HG-34-II, sulfated human big gastrin; PG-17-I, nonsulfated porcine little gastrin; PG-17-II, sulfated porcine little gastrin.

the relation between acid secretion rates and change in serum immunoreactive gastrin.

METHODS

Gastrins. All gastrin preparations were generous gifts from Professor R. A. Gregory and Dr. Hilda Tracy, University of Liverpool. Porcine gastrins, PG-17-I and PG-17-II, were obtained from extracts of hog antral mucosa (10). Natural human gastrins, HG-34-I, HG-34-II, and HG-17-I were obtained from gastrin-secreting tumors of the Zollinger-Ellison variety. Each peptide was purified by AE-cellulose chromatography and column electrophoresis (11). The purity of each of the gastrin preparations was proved by homogeneity in these systems and by quantitative amino acid analysis.

Animals. Four mongrel dogs weighing between 20 and 26 kg were prepared with gastric fistulas drained by Thomas cannulas (12) and Heidenhain pouches drained by Gregory cannulas (13). Experiments were performed at least 3 mo after operation. The dogs were deprived of food but not water for 18 h before each test. Experiments were performed no more than twice per week.

The maximal acid secretory capacity of each pouch was determined by stimulation with pentagastrin (Ayerst Laboratories, New York) at doses of 8 and 16 μg/kg-h. Each dose was given on separate days and each dog was studied twice. The highest rate of acid secretion obtained for three consecutive 10-min periods was taken as the maximal pentagastrin response. Acid secretory rates were determined as previously described (14) by titration to pH 7 on an autotitrator (Autoburette, Radiometer Co., Copenhagen). All other acid secretion rates were expressed as percent maximal pentagastrin-stimulated secretion to minimize variations due to differences in the sizes of the pouches.

Gastrin solutions were prepared in 0.05 M NH_4HCO_3. Absorbance at 280 nm was measured in a Zeiss PMQ-II spectrophotometer (Carl Zeiss, Inc., New York) and gastrin concentrations were calculated from the molar extinction coefficients. G-17 and G-34 have identical C-terminal heptadecapeptide composition and the N-terminal heptadecapeptide portion of G-34 contains no tryptophan or tyrosine (R. A. Gregory, personal communication). Therefore the extinction coefficients for G-17 and G-34 are identical. Sulfation of tyrosine decreases the extinction coefficient of tyrosine from 1,507 to less than 1 (15), thus coefficients for gastrins I and II differ. The calculated molar extinction coefficient in alkaline solution for PG-17-I, HG-17-I, and HG-34-I is 12,261 and for PG-17-II and HG-34-II is 10,754 (16). The molecular weights of these compounds are: PG-17-I, 2,116; HG-17-I, 2,098; PG-17-II, 2,196; HG-34-I, 3,839; and HG-34-II, 3,919.

Infusions of gastrin solutions were given for 90 min intravenously by use of a Harvard syringe pump (Harvard Apparatus Co., Inc., Millis, Mass.). Only one dose of one gastrin preparation was given per day. In separate studies, PG-17-I and HG-34-I, PG-17-II and HG-34-II, and PG-17-I and HG-17-I were compared. Each week two dogs received one dose of a pair of stimulants and the other two dogs received the same dose of the other member of the pair; on the 2nd study day of the week the hormones were reversed. The dose range covered was 25–300 pmol/kg-h. The number of studies performed was limited by the supply of each peptide available so that only one set was performed for each form of gastrin.

Before gastrin infusion, gastric fistula and Heidenhain pouch secretions were collected for 30 min and two blood samples were obtained for serum gastrin determination. Acid secretion was measured in consecutive 10-min periods during infusions of gastrin and afterward until acid secretion had returned to basal rates. 5-ml blood samples were obtained every 15–30 min during infusion and at 2- to 5-min intervals after infusion was discontinued. As many as 22 samples were obtained from each dog. Serum was separated and stored at −20°C. All samples obtained during a study with a pair of peptides were measured by radioimmunoassay at the same time. Acid responses were taken as the highest three consecutive 10-min outputs for each dose and each output was normalized by dividing by the peak 30-min pentagastrin output and multiplying the result by 100.

Steady-state serum gastrin values were taken as the mean of the last two values obtained during infusion. For determination of half time, basal gastrin concentration was subtracted from all values. All values for each dog were normalized by expressing them as a percentage of steady-state value; then the mean of normalized values of the four dogs was taken for each time period, converted to the natural logarithm, and regression of ln serum gastrin vs. time was computed to yield the slope k_e from which the disappearance half time was determined by dividing into 0.693. Clearance rate was determined by dividing the infusion rate in picomoles per kilogram per minute by the steady-state increment in serum gastrin in picomoles per liter for the two or three highest doses and expressed as an average in liters per kilogram per minute.

Space of distribution was calculated by use of the plateau principle described by Goldstein, Aronow, and Kalman (17) from the formula:

$$V = D/[(G)(k_e)],$$

where V = volume of distribution as fraction of body weight, D = dose of gastrin expressed as picomoles per kilogram per minute, G = steady-state blood level of gastrin expressed as picomoles per liter, and k_e = elimination constant.

Serum gastrin measurements were done by radioimmunoassay as previously described (3). The antibody used for these assays was no. 2604, donated by Dr. Jens Rehfeld, Copenhagen (18), and was used at a final dilution of 1:120,000. Natural HG-17-I was labeled with ^{125}I and repurified by starch gel electrophoresis (2). Approximately 1,600 cpm of labeled gastrin were used in each 2-ml incubation, giving a concentration of labeled gastrin less than 1 pg/ml in the incubation. Gastrin solutions used for infusion were saved and used for radioimmunoassay standards and for determination of relative immunopotency. Separation was done with ion-exchange resin IRP 58M (2).

Immunoreactivity of G-17 and G-34. Preliminary experiments were done to determine the immunochemical potency of HG-34-I and HG-34-II compared with the corresponding human and porcine heptadecapeptides. When solutions were made equimolar by use of the molar extinction coefficient and measured absorbance at 280 nm, G-17 preparations consistently were about 1.5 times more potent in inhibiting the binding of labeled gastrin to antibody 2604. This was true of both sulfated and nonsulfated forms (Figs. 1 and 2). To validate this difference in immunopotency of G-34 and G-17, trypsinization experiments were performed. In these experiments, solutions of HG-17-I, HG-34-I, and HG-34-II were incubated with a dilute solution of trypsin (Schwarz Mann Div., Orangeburg, N. Y., lot Y 1458, 3050 N.F. U/mg) and samples were removed at specified time intervals. Each sample was assayed with antibody 2604 and with our own antibody 1295 which has a high degree

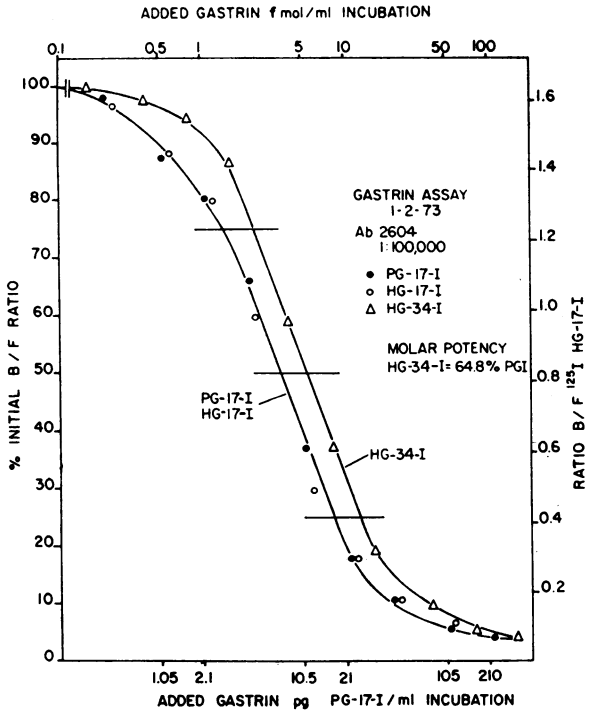

FIGURE 1 Standard curves obtained with antibody 2604. Comparison of immunochemical potency of nonsulfated porcine (PG-17-I) and human (HG-17-I) G-17 and nonsulfated human G-34. Potency ratio computed by average of molar concentration of G-17 and of G-34 required to produce, 25, 50, and 75% inhibition of initial ratio of bound to free (B/F)-labeled gastrin. Molar concentration of gastrin in the incubation mixture in indicated at the top. At the bottom the concentrations are expressed in equivalent weights of PG-17-I, thus understanding the amount of G-34 present.

of specificity for G-17 compared with G-34. The principle upon which these studies were based is the observation that G-34 is converted by trypsin to G-17 and one or more N-terminal peptides (9). Results are shown in Table I and in Fig. 3. Addition of trypsin resulted in generation of additional gastrin immunoreactivity from HG-34-I and HG-34-II but not from HG-17-I. The change was more dramatic with antibody 1295 but was also clear-cut with antibody 2604. The conversion reaction appeared to be complete within 10 min. With antibody 2604 the measured increase in immunoreactivity for HG-34-I was 43% and 45% for HG-34-II. These results with trypsinization confirmed the original observation that there was approximately a 3:2 ratio of immunopotency between G-17 and G-34 with this antibody. An average figure of 44% was taken and all serum results obtained for HG-34-I and -II were multiplied by a conversion factor of 1.44 after being read from a PG-17-I or -II standard curve. The molar concentrations calculated in this way differed by less than 10% from those obtained by reading directly from a G-34 standard curve.

RESULTS

Base-line values. Basal serum gastrin concentrations and basal and peak 30 min pentagastrin-stimulated acid secretion rates are shown in Table II. Serum gastrin concentrations 30–60 min after cessation of infusion of the various gastrins did not differ significantly from basal values obtained before infusion.

Serum immunoreactive gastrin during and after gastrin infusions. For each molar dose, G-34 preparations produced higher blood levels of gastrin than G-17 preparations. Results of infusion with HG-34-II and PG-17-II are shown in Fig. 4. Increment in serum gastrin was linearly related to dose of gastrin (Table III) as would be expected if removal rate and space of distribution did not vary with dose. The Y intercept of the regression lines did not differ significantly from zero (except for HG-34-I) as would be expected from the hypothesis that there should be no increment in serum gastrin when the dose is zero. For both G-17 and G-34, the slopes for the sulfated forms did not differ significantly from those of the unsulfated forms. The slope of the G-34 forms was 5.8 times greater than that for the G-17 forms indicating that with equimolar rates of infusion the serum increment was almost 6 times greater with G-34 than with G-17.

Half-time studies are shown in Fig. 5. These data were obtained immediately after cessation of the 200 or 300 pmol/kg-h doses. The data are normalized so that 100 represents the steady-state concentration minus basal

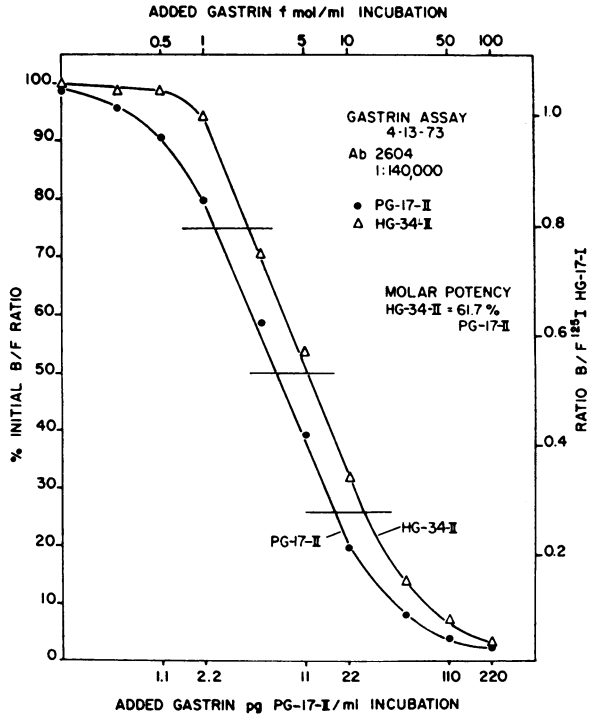

FIGURE 2 Standard curves for comparison of immunochemical potency of sulfated PG-17 and HG-34. Antibody and units identical with Fig. 1.

TABLE I

Effect of Trypsinization on Immunoreactivity of HG-34 and HG-17 with Antibodies 2604 and 1295

Sample	Ab 2604 1:120,000			Ab 1295 1:15,000		
	HG-17-I	HG-34-I	HG-34-II	HG-17-I	HG-34-I	HG-34-II
B1	30.8*	7.9*	4.7*	33.1*	1.2*	0.7*
B2	32.1	9.6	5.0	32.0	1.0	0.3
B3	30.4	7.2	4.4	30.9	1.1	0.8
1 min	32.4	11.1	6.1	31.5	4.5	1.3
2 min	34.8	14.3	7.1	34.4	9.0	4.8
5 min	31.6	12.7	6.7	34.0	10.1	6.2
10 min	33.3	11.3	6.6	34.4	12.2	6.5
20 min	29.8	11.1	6.6	30.3	11.8	7.8
40 min	31.8	11.6	7.2	33.4	12.2	6.6
Mean basal	31.1	8.2	4.7	32.0	1.1	0.6
Mean 5–40 min	31.6	11.7	6.8	33.0	11.6	6.8
5–40 min/basal	1.02	1.43	1.45	1.03	10.55	11.33

* All concentrations expressed as picomoles per milliliter vs. HG-17-I standard.

near the end of the infusion period. In all cases the logarithm of the normalized concentration plotted against time gave good fit on linear regression. It is apparent that the half time of G-34 was four to six times longer than that of G-17.

Space of distribution was calculated from the elimination constant (k_e) obtained during the half-life measurement and from the observed increments in serum gastrin during infusion of the three highest doses. The clearance rates also were calculated from the same data. Results are shown in Table IV. In these dogs the half times of the G-17 preparations were between 2.7 and 3.9 min and for the G-34 preparations between 14.7 and 16.8 min. The mean ratio of half times of G-34 and G-17 was 4.9 which agrees satisfactorily with the mean ratio of slopes of regression of serum gastrin increment on dose, 5.8. These two ratios are independent estimates of relative removal rates. Space of distribution was similar for all preparations, ranging from 22 to 28% body weight. Clearance rate for G-17 was six times greater than for G-34.

Acid secretion vs. infusion rate and change in serum gastrin. Complete results of all infusion studies in individual dogs are given in Table V. For equimolar rates of infusion, the acid secretory rates produced by G-34 were modestly greater than those produced by

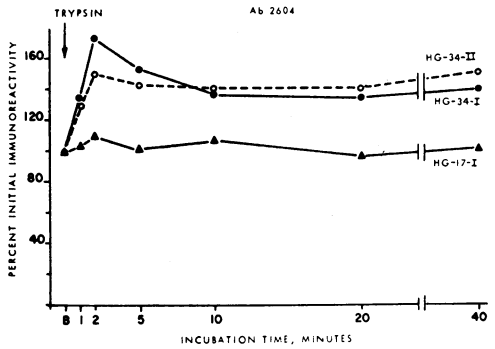

FIGURE 3 Time curves of change in immunochemical gastrin concentration after introduction of trypsin, 0.010 mg/ml, into solutions of G-34-I and G-34-II. A solution of G-17-I served as a control for recovery and for losses due to chymotrypsin contamination. B represents the mean value obtained in three samples removed from the reaction vial before addition of trypsin. Incubations were done in 0.1 M phosphate buffer at 22°C. 2-ml aliquots were removed and heated at 100°C for 5 min to inactivate trypsin. Each solution was tested for immunoreactive gastrin concentration with antibody 2604.

TABLE II

Base-Line Serum Gastrin and Peak Acid Secretory Rates

	Dog A	Dog B	Dog C	Dog D
Mean fasting gastrin, *fmol/ml*	14.6	29.0	19.9	23.6
Basal acid secretion, *μeq/30 min*				
Gastric fistula	18	828	240	0
Heidenhain pouch	0	0	0	0
Peak acid secretion during maximal stimulation with pentagastrin, *μeq/30 min*				
Gastric fistula	18,782	20,601	19,164	12,929
Heidenhain pouch	441	1,040	834	2,851

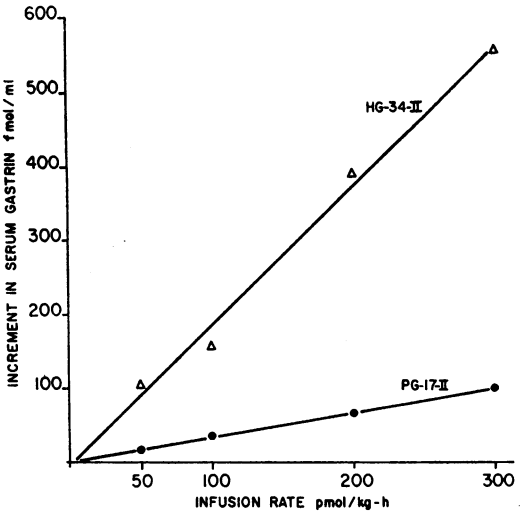

FIGURE 4 Increments in serum immunoreactive gastrin (plateau values during infusion minus basal concentration) as a function of molar infusion rates of PG-17-II and HG-34-II in four dogs.

G-17. However, increments in serum gastrin were much greater during infusion of the same molar doses of the larger types of gastrin (Fig. 6).

The comparability of HG-17-I and of PG-17-I is shown in Fig. 7. There was no significant difference in potency as stimulants of acid secretion, and as already

TABLE III

Parameters for Linear Regression of Increments in Serum Gastrin on Dose of Gastrin

Kind of gastrin	*Y* intercept *fmol/ml* $a \pm SE$	Slope *(fmol/ml)/(fmol/kg-h)* $b \pm SE$	Correlation coefficient *r*
PG-17-I	−9.4±3.4	0.34±0.02	0.996
PG-17-II	−1.6±1.3	0.34±0.01	0.999
PG-17-I + PG-17-II	−6.5±3.3	0.35±0.02	0.991
HG-34-I	−42.4±16.0	2.53±0.14	0.997
HG-34-II	−6.6±24.1	1.89±0.13	0.995
HG-34-I + HG-34-II	−10.7±24.5	2.02±0.16	0.982

shown there was no difference in half time. Thus, it appears that PG-17-I can be used interchangeably with HG-17-I in comparative studies of biological activity. (This fact was important in the present studies because tests were done before pure natural HG-17-I became available in amounts sufficient for testing.)

Figs. 8–11 illustrate the potency of G-17 vs. G-34 in stimulation of acid secretion from gastric fistulas and Heidenhain pouches. The data are expressed in two different ways: as a function of exogenous dose and as a function of increment in serum gastrin. With both types of stomach preparation there was a marked difference in potency of circulating G-34 and G-17. To produce the same rate of acid stimulation, approximately five

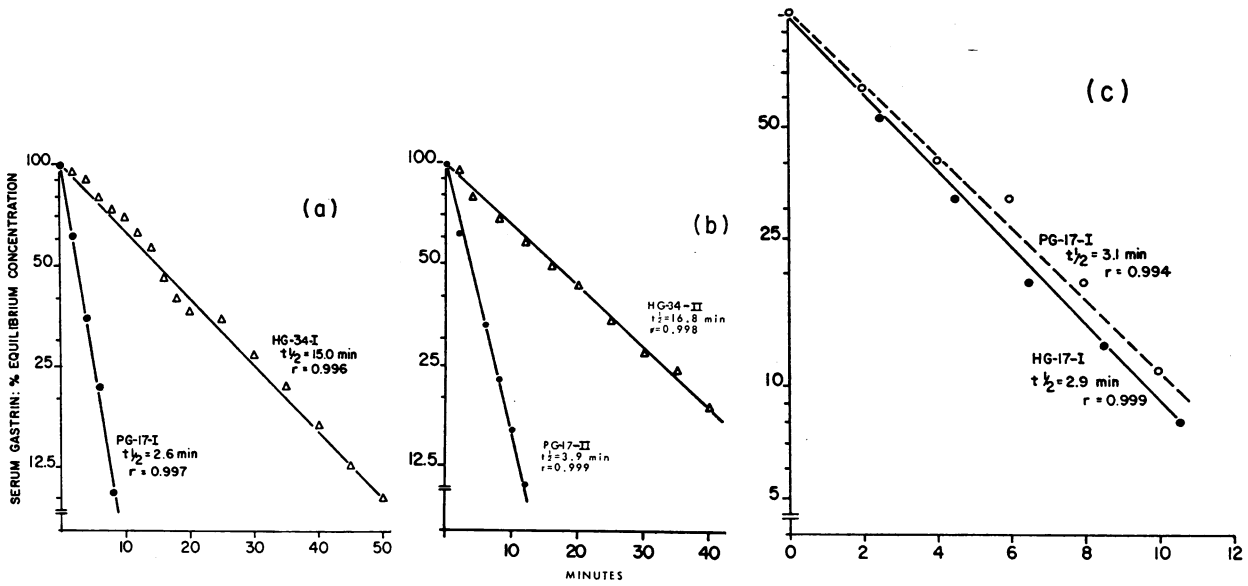

FIGURE 5 Half-time determinations obtained when intravenous infusions of gastrin were stopped. Equilibrium concentration equals steady-state concentration after basal gastrin has been subtracted. Values are normalized so that equilibrium concentration equals 100%. Half time was calculated from elimination constants obtained by linear regression analysis of natural logarithm of normalized concentration vs. time. Three separate determinations were done for comparison of pairs of gastrins; each measurement was performed in four dogs: (*a*) PG-17-I vs. HG-34-I, (*b*) PG-17-II vs. HG-34-II, and (*c*) PG-17-I vs. HG-17-I.

TABLE IV

Half Time, Space of Distribution, and Clearance Rate of G-17 and G-34

	Half time	Space of distribution	Clearance rate
	min	*liters/kg*	*ml/kg-min*
PG-17-I	2.7	0.23	57.9
PG-17-II	3.9	0.28	52.5
HG-17-I	3.1	0.24	—
HG-34-I	14.7	0.23	9.1
HG-34-II	16.8	0.22	9.1

times as great an increment of serum G-34 was required as for G-17. With equimolar exogenous doses, the increase in acid secretion was about 20% higher with HG-34 than with G-17. The molar doses of exogenous G-17 and G-34 required to produce equal rates of acid secretion would produce four to five times higher increments in circulating G-34 than in G-17. Thus, based on exogenous dose G-34 is 1.4 times more potent than G-17, but based on circulating concentration G-17 is about 5 times more potent than G-34.

DISCUSSION

The present studies indicate important biological differences between G-17 and G-34. Previous studies in which acid secretion has been related to changes in serum gastrin concentration must be interpreted with some caution, since the specific molecular types of gastrin in the circulation rarely have been determined.

The present half-time determinations for G-17 are in good general agreement with other workers. The half time of synthetic human gastrin measured directly after intravenous infusion in dogs was between 2 and 4 min (19, 20) and was slightly longer when estimated by plateau concentrations achieved during intravenous infusion (21). Straus and Yalow found half times of 3 and 9 min for G-17 and G-34, respectively after rapid intravenous injection (22) and estimated initial spaces of distribution approximately half as great as those found during prolonged infusion during the present investigation. The differences may reflect variations in the methods of administration or may simply represent biological variations among the small numbers of dogs used in each investigation.

Measurement of maximal responses to gastrin in dogs is difficult because supramaximal doses of gastrin cause inhibition of acid secretion in the dog and the dose at which such inhibition occurs varies among dogs. At any given dose of gastrin administered to a group of dogs some may be submaximally and some maximally stimulated while others are partially inhibited. However, from the data obtained in the present investigation it appears that G-17 and G-34 produce similar maximal acid responses and that these are in turn similar to the maximal response to pentagastrin.

TABLE V

Changes in Serum Gastrin and Acid Secretion during Gastrin Infusion at Different Rates

Gastrin		Increment in serum gastrin				Gastric fistula acid secretion				Heidenhain pouch acid secretion			
Type	Dose	Dog A	Dog B	Dog C	Dog D	Dog A	Dog B	Dog C	Dog D	Dog A	Dog B	Dog C	Dog D
	pmol/kg-h	*fmol/ml*				*% maximal pentagastrin*				*% maximal pentagastrin*			
PG-17-II	25	—	—	—	—	18	1 8	9	18	19	2	8	2
PG-17-I	35	5	6	7	6	18	26	19	1	26	3	6	2
PG-17-II	50	15	15	12	16	26	32	45	32	6	10	23	42
PG-17-I	70	16	9	7	8	30	24	36	5	36	7	24	12
PG-17-II	100	33	52	13	37	50	62	40	56	31	68	7	61
PG-17-I	140	40	32	44	36	57	73	51	51	109	36	57	105
PG-17-II	200	60	52	86	64	78	72	100	88	49	60	101	124
PG-17-I	280	114	81	63	86	94	74	90	58	50	73	43	86
PG-17-II	300	119	93	92	98	75	91	66	96	57	54	34	89
HG-34-I	25	32	26	20	32	8	23	21	3	6	3	12	13
HG-34-II	25	—	—	—	—	8	25	21	7	9	8	24	2
HG-34-I	50	63	69	59	79	28	54	30	19	30	37	28	32
HG-34-II	50	63	102	125	115	34	48	51	57	21	26	30	72
HG-34-I	100	189	228	266	226	49	71	46	52	79	54	40	74
HG-34-II	100	194	148	154	130	48	71	67	59	26	54	33	86
HG-34-I	200	419	448	442	528	87	85	98	71	44	89	65	113
HG-34-II	200	321	367	544	325	94	93	98	137	56	81	36	140
HG-34-II	300	667	421	472	658	109	117	83	122	52	49	54	163

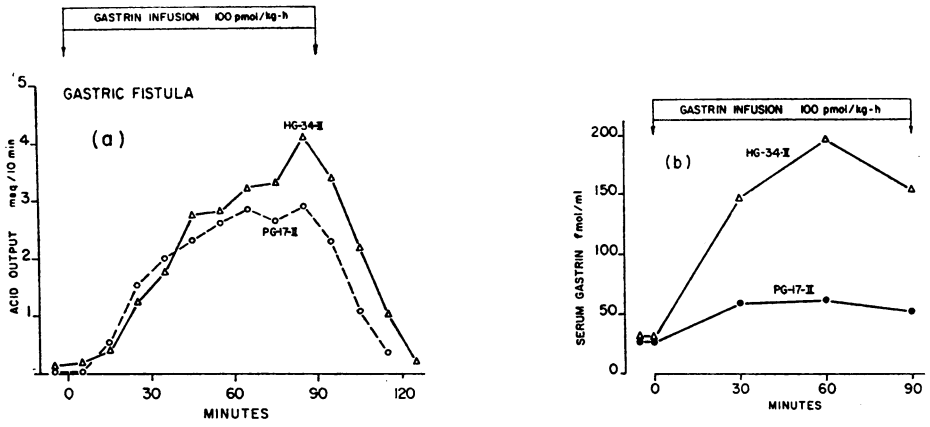

FIGURE 6 Comparison of changes in acid secretion rate and increments in serum gastrin during infusion of 100 pmol/kg-h dose of PG-17-II and HG-34-II. Gastric fistula acid secretion is indicated as (*a*) and immunoreactive serum gastrin as measured with antibody 2604 is shown as (*b*) (values above basal during HG-34-II infusion have been multiplied by 1.44 to correct for the difference in immunochemical potency between HG-34-II and PG-17-II in this antibody).

Our results indicate that under steady-state conditions approximately five times higher molar concentrations of G-34 than of G-17 in the circulation are required to produce equal rates of acid secretion. It is possible that G-34 must be converted to G-17 at the receptor before producing its response. Such a possibility is not supported by Straus and Yalow's finding that there was no appearance of G-17 in the plasma after single rapid injections of G-34 (22). Further studies are needed to determine whether G-17 appears in plasma after prolonged infusion of G-34. Because the spaces of distribution of G-34 and G-17 are similar during infusions, it is likely that concentrations of these hormones at the receptor sites are reflected by their concentrations in the serum. Such equilibration of serum and receptor compartments may not pertain to changes in serum gastrin produced by rapid injections of gastrin or release of large amounts of gastrin into the circulation quickly such as may occur immediately after ingesion of a meal or in patients with Zollinger-Ellison syndrome after an intravenous bolus of secretion (23).

The potency of gastrin can be defined either in terms of exogenous dose or of serum concentration or endogenous dose. Compounds which produce equimolar increments in serum concentration when infused at equimolar rates and which have equal efficacy (identical maximal response) should behave identically whether exogenous dose or change in serum concentration is measured. PG-17-I, PG-17-II, and HG-17-I meet these criteria when they are intercompared as do HG-34-I and II when they are compared only with each other.

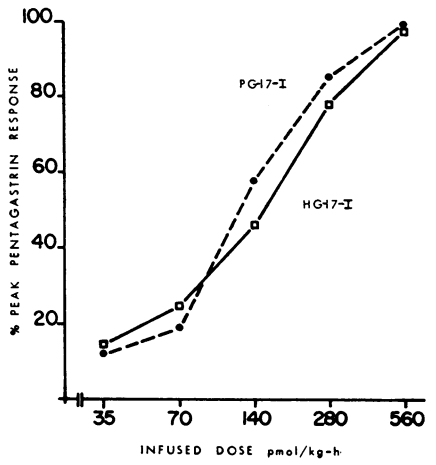

FIGURE 7 Dose-response curves obtained with equimolar doses of HG-17-I and PG-17-I in four dogs with gastric fistula.

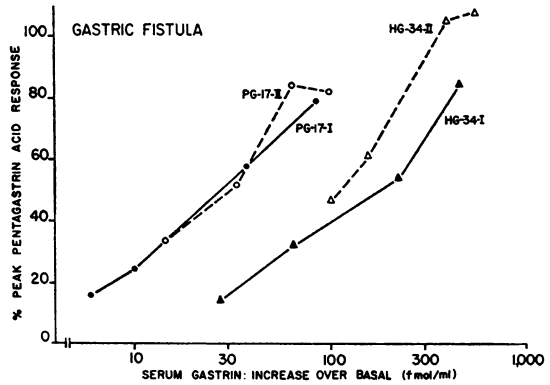

FIGURE 8 Relationship between increase in serum immunoreactive gastrin (corrected for differences in immunoreactivity) and gastric fistula acid secretion.

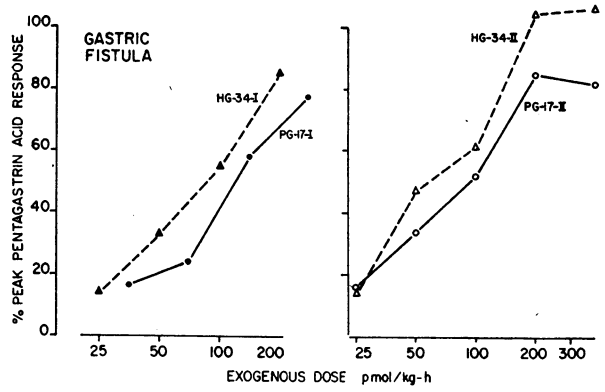

FIGURE 9 Relationship between gastric fistula acid secretion and exogenous dose of two preparations of HG-17 and two preparations of HG-34.

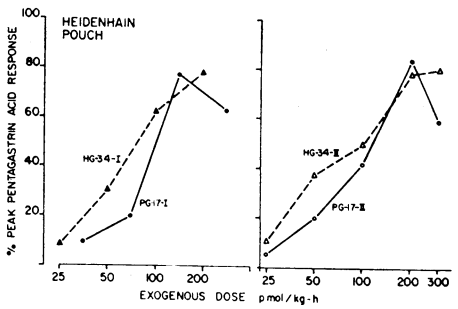

FIGURE 11 Relationship between Heidenhain pouch acid secretion and exogenous dose of two preparations of PG-17 and two preparations of HG-34.

However, G-17 and G-34 compounds cannot be directly intercompared. If only exogenous doses are compared, G-17 and G-34 appear to have similar potencies, with G-34 being slightly more potent. But when circulating concentrations, or "endogenous doses" are compared, G-17 is found to be much more potent than G-34. The latter type of potency is more directly applicable to measurements of serum gastrin concentration, where circulating concentrations of hormone are measured but endogenous secretion rates of the gastrins, corresponding to infusion rates, can only be inferred. Durkin and Kucera (24) reported that partially purified hog big gastrin was more potent than hog little gastrin when exogenous immunochemical doses were compared and that big gastrin produced higher blood levels. However, they did not establish the specificity of their antibody, so the chemical amounts of big and little gastrin which they administered are unknown.

Molecular forms of gastrin different from G-17 and G-34 are known to be present in the circulation. Yalow (25) has identified a "big-big" form of gastrin which predominates in fasting plasma and found that the half time of a similar gastrin fraction obtained from a Zollinger-Ellison tumor was approximately 90 min (22). The biological activity of big-big gastrin is not known. Rehfeld has identified gastrin immunoreactivity which elutes between big-big gastrin and G-34 (7). The biological activity of this material, known as "component I" also remains to be determined. A smaller molecular form of gastrin, "minigastrin" (G-13, the C-terminal tridecapeptide of G-17), also has been found to comprise a small fraction of serum immunoreactive gastrin (7). The exogenous potency of minigastrin in the dog is approximately half that of G-17 and the half-life is similar to that of G-17 (26).

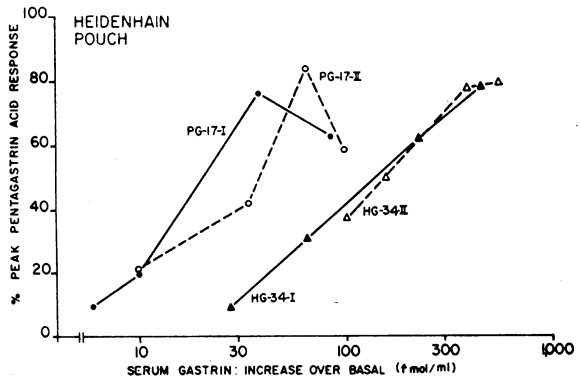

FIGURE 10 Relationship between Heidenhain pouch acid secretion and increment in serum immunoreactive gastrin (corrected for differences in immunoreactivity).

The measured gastrin concentration in the circulation may vary according to the immunochemical specificities of the antibodies employed in radioimmunoassay. Most assay systems have been defined by the inhibition curve produced with a G-17 standard and have not considered possible immunochemical differences between G-17 and G-34 or other circulating forms of gastrin. Yalow (6) and Rehfeld (18) have reported similar inhibition with G-17 and G-34 in their systems. Such antibodies should be useful in determination of total immunoreactive serum gastrin regardless of molecular species but may provide misleading estimates of acid-stimulating action if there is a predominance of a single form. Antibodies which detect a single molecular size of gastrin and have minimal reactivity with other sizes might be used in combination to define the molecular pattern of gastrin in the serum. Antibody 1295 appears to be highly specific for G-17 (26), but antibodies specific for G-34 have not yet been reported. Hansky (27) reported an antibody which differentiates between sulfated and nonsulfated gastrins. We have produced antibodies with similar specificities (26). In addition, some antibodies, especially those produced by immunization of guinea pigs with crude gastrin distinguish amino acid substitutions in the G-17 molecule among species (26). With such antibodies pure gastrin from the appropriate species

must be used as the standard to obtain accurate estimates of gastrin concentration.

Until antibodies of known specificities are available which clearly distinguish the different forms of gastrin, the best separation method available is fractionation by size, charge, or both (4, 8) followed by assay of each fraction with a broadly reactive antibody. Such fractionation studies may permit some as yet unrecognized distinctions between normal subjects and those with gastric acid hypersecretion and/or peptic ulcer disease. For example, if it were found that some persons have a predominance of G-17 rather than big-big gastrin in their fasting serum, this could account for basal acid hypersecretion with normal total serum gastrin concentrations.

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