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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.

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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.

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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 shown 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...
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). Each dog was given a 3-day preparatory period of no tryptophan or tyrosine diet, 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 mg/kg-h. Each dog was given five separate 10-min infusions of the two or three highest doses and each dose was taken for each time period, converted to the natural logarithm, and regression of 

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 incubation 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-I and G-34-II, 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 of

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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
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 equinolar rates of infusion, the acid secretory rates produced by G-34 were modestly greater than those produced by
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 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](image-url)  
**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 III**  
Parameters for Linear Regression of Increments in Serum Gastrin on Dose of Gastrin

<table>
<thead>
<tr>
<th>Kind of gastrin</th>
<th>Slope (fmol/ml)</th>
<th>Intercept (fmol/ml)</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG-17-I</td>
<td>-9.4 ± 3.4</td>
<td>0.34 ± 0.02</td>
<td>0.996</td>
</tr>
<tr>
<td>PG-17-II</td>
<td>-1.6 ± 1.3</td>
<td>0.34 ± 0.01</td>
<td>0.999</td>
</tr>
<tr>
<td>PG-17-I + PG-17-II</td>
<td>-6.5 ± 3.3</td>
<td>0.35 ± 0.02</td>
<td>0.991</td>
</tr>
<tr>
<td>HG-34-I</td>
<td>-42.4 ± 16.0</td>
<td>2.53 ± 0.14</td>
<td>0.997</td>
</tr>
<tr>
<td>HG-34-II</td>
<td>-6.6 ± 24.1</td>
<td>1.89 ± 0.13</td>
<td>0.995</td>
</tr>
<tr>
<td>HG-34-I + HG-34-II</td>
<td>-10.7 ± 24.5</td>
<td>2.02 ± 0.16</td>
<td>0.982</td>
</tr>
</tbody>
</table>

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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 IV

<table>
<thead>
<tr>
<th>Halftime</th>
<th>Space of distribution</th>
<th>Clearance rate</th>
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<tr>
<td>2.7</td>
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<td>57.9</td>
</tr>
<tr>
<td>3.9</td>
<td>0.28</td>
<td>32.5</td>
</tr>
<tr>
<td>3.1</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>14.7</td>
<td>0.23</td>
<td>9.1</td>
</tr>
<tr>
<td>16.8</td>
<td>0.22</td>
<td>9.1</td>
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### TABLE V

<table>
<thead>
<tr>
<th>Gastrin Type</th>
<th>Increment in serum gastrin</th>
<th>Gastric fistula acid secretion</th>
<th>Heidenhain pouch acid secretion</th>
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</thead>
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<tr>
<td></td>
<td>Dog A</td>
<td>Dog B</td>
<td>Dog C</td>
</tr>
<tr>
<td>PG-17-II</td>
<td>25</td>
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<tr>
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<td>6</td>
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<tr>
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<tr>
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<td>9</td>
</tr>
<tr>
<td>PG-17-II</td>
<td>100</td>
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<td>52</td>
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<tr>
<td>PG-17-I</td>
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<td>32</td>
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<tr>
<td>PG-17-II</td>
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<tr>
<td>PG-17-I</td>
<td>280</td>
<td>114</td>
<td>81</td>
</tr>
<tr>
<td>PG-17-II</td>
<td>300</td>
<td>119</td>
<td>93</td>
</tr>
<tr>
<td>HG-34-I</td>
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<td>26</td>
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<tr>
<td>HG-34-II</td>
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<td>69</td>
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<tr>
<td>HG-34-I</td>
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<td>102</td>
</tr>
<tr>
<td>HG-34-II</td>
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<td>189</td>
<td>228</td>
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<tr>
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<td>194</td>
<td>148</td>
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<tr>
<td>HG-34-II</td>
<td>200</td>
<td>419</td>
<td>448</td>
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<tr>
<td>HG-34-I</td>
<td>200</td>
<td>321</td>
<td>367</td>
</tr>
<tr>
<td>HG-34-II</td>
<td>300</td>
<td>667</td>
<td>421</td>
</tr>
</tbody>
</table>

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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 ingestion 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.
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 immunocompounds 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 tri-decapeptide 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).

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 immunocompound 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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