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# CHINCHILLA "BIG" AND "LITTLE" GASTRINS

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**SUMMARY:** Gastrin heptadecapeptides (gastrins I and II which differ in the presence of sulfate on the tyrosine of the latter) have been purified and sequenced from several mammalian species including pig, dog, cat, sheep, cow, human and rat. A 34 amino acid precursor ("big" gastrin), generally accounting for only 5% of total gastrin immunoreactivity, has been purified and sequenced only from the pig, human, dog and goat. Recently we have demonstrated that guinea pig (GP) "little" gastrin is a hexadecapeptide due to a deletion of a glutamic acid in the region 6-9 from its $NH_2$-terminus and that GP "big" gastrin is a 33 amino acid peptide. The chinchilla, like the GP, is a New World hystricomorph. This report describes the extraction and purification of "little" and "big" gastrins from 31 chinchilla antra. Chinchilla "little" gastrin is a hexadecapeptide with a sequence identical to that of the GP and its "big" gastrin is a 33 amino acid peptide with the following sequence: <ELEPQGPPHLGTDLSKKQGPWAEEEAAYGWMDF# 

Gastrin heptadecapeptides (gastrins I and II which differ in the presence of sulfate on the tyrosine of the latter) have been purified and sequenced from several mammalian species including pig (1), dog, cat, sheep, cow (2), human (3), rat (4), dog and goat (5). A 34 amino acid precursor ("big" gastrin), generally accounting for only 5% of antral gastrin immunoreactivity (6), has been purified and sequenced from the pig, human (7), dog and goat (5). Recently we have demonstrated that guinea pig (GP) "little" gastrin is a hexadecapeptide due to a deletion of a glutamic acid in the region 6-9 from its $NH_2$-terminus (8) and its "big" gastrin is a 33 amino acid peptide (9). Since the chinchilla, like the GP, is a New World hystricomorph it was of interest to determine whether its gastrins would resemble those of the GP or

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**ABBREVIATIONS:** GP, guinea pig; IR, immunoreactivity; ME, mercaptoethanol; ACN, acetonitrile; TFA, trifluoroacetic acid; PAP, pyroglutamyl aminopeptidase.

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of the Old World mammals. In this report we describe the extraction and purification on HPLC of chinchilla "little" and "big" gastrins from the antra of 31 animals and demonstrate that these gastrins resemble those of the GP.

## MATERIALS AND METHODS

Chinchilla carcasses were frozen after the pelts were removed at Chinchilla of Catskill, Catskill, NY and at the Hykes Chinchilla Ranch, Waynesboro, PA. Following partial thawing in our laboratory the gastrointestinal tracts were removed. The distal third of 31 stomachs was removed, washed in acetone and then pooled to yield a total weight of 16.7g.

Radioimmunoassay (RIA): Gastrin immunoreactivity (IR) was monitored with a RIA employing an antibody (R71) that recognizes the gastrin COOH-terminus (10,11). Human antral gastrin (gift of Dr. R.A. Gregory) was used for labeling with $^{125}I$ and synthetic human gastrin (Peninsula Laboratories, Belmont, CA) was used as standard.

Extraction and Purification: The distal stomachs were boiled in $H_2O$ for 5 minutes, cooled and then extracted in 5 volumes of 0.1M $NH_4HCO_3$ containing 0.2% mercaptoethanol (ME). The suspension was centrifuged at 3000g for 1 hour at 4°C. The supernatant was further clarified by addition of an equal volume of acetone and storage overnight at -20°C. The precipitate that formed was removed by centrifugation. The extract was then pumped through a 50 ml column of QMA anion exchange silica (Waters Associates) at a flow rate of 50 ml/hr and batch eluted at the same flow rate with 0.05M Tris-HCl, pH7, (Tris) containing 1M NaCl. The elution fractions containing the peak gastrin-IR were pooled and then purified by several HPLC steps using the following sequence of columns and linear gradient elution conditions.

1. μBondapak $C_{18}$ radial pak cartridge (Waters Associates) eluted with 0-30% acetonitrile (ACN) in Tris. There were four peaks of gastrin-IR (A,B,C,D). Each was separately applied to the same columns with the same elution conditions.
2. Mono Q HR5/5 strong anion exchange column (Pharmacia) eluted with 0-0.3 M NaCl in Tris/20% ACN.
3. Nova $C_{18}$ radial pak cartridge (Waters Associates) eluted with 20-40% ACN in 0.1% trifluoroacetic acid (TFA). HPLC flow rates were 1 ml/min except the μBondapak step which was 2 ml/min.

Amino Acid Analysis and Sequencing: A portion of the purified Peak A (140 pmol) was hydrolyzed in 50 μl 2M NaOH in capped polyethylene microfuge tubes at 105°C for 16 hours. The hydrolysate was neutralized with 2M HCl, an aliquot was derivatized with o-phthalaldehyde and amino acids were identified by HPLC with fluorescence detection (12).

The COOH-terminal phenylalanine α-amide of the purified Peak A was determined by a modification of the method described by Tatemoto and Mutt (13), as previously reported (5).

The $NH_2$-terminus of each peptide was enzymatically deblocked with pyroglutamyl aminopeptidase (PAP, Sigma Chemical Co) (14) at a ratio of 1 unit PAP/nmol "little" gastrin (A and B) and 8 units PAP/nmol "big" gastrin (C and D) in a volume of 200 μl. Deblocked peptides were repurified on a Nova $C_{18}$ column using the elution conditions employed in step 3. Automated amino acid sequencing of the PAP-treated peptides (20-100 pmol) was performed using a gas phase sequencer (15). Phenylthiohydantoin derivatives of amino acids were identified with an on-line analyzer.

## RESULTS

The antiserum used in the RIA is directed towards the COOH-terminus of gastrin and a dilution curve of the chinchilla antral extract was superposable along the human gastrin standard curve. There was a total of 26 nmol in the original extract; the recovery after concentration on the anion exchange column step was 45%. The first purification step is shown in Fig. 1. Peaks A and B are the "little" gastrins; Peaks C and D are the "big" gastrins. The minor peak eluting earlier than Peak A was not identified. In this system the sulfated gastrin elutes earlier than the unsulfated form (8) so that Peak A corresponds to "little" gastrin II. The subsequent purification steps for the individual peaks are shown in Figs. 2 and 3. Since there were 6 nmol of Peak A and 2.4 nmol of Peak B available after step 1, the two further steps of

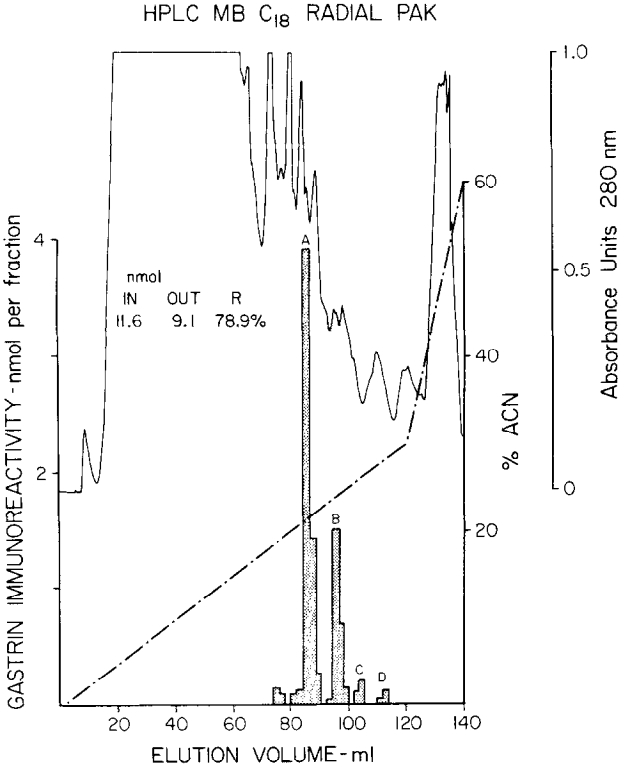


Fig. 1. First HPLC step in purification of chinchilla gastrins. The column was eluted with 0-30% acetonitrile (ACN) in 0.05 M Tris-HCl pH7 (Tris). Peaks A and B are the sulfated and non-sulfated "little" gastrins, respectively, and peaks C and D are the sulfated and non-sulfated "big" gastrins, respectively. The earliest eluting peak was not identified. In this and subsequent figures R signifies recovery.

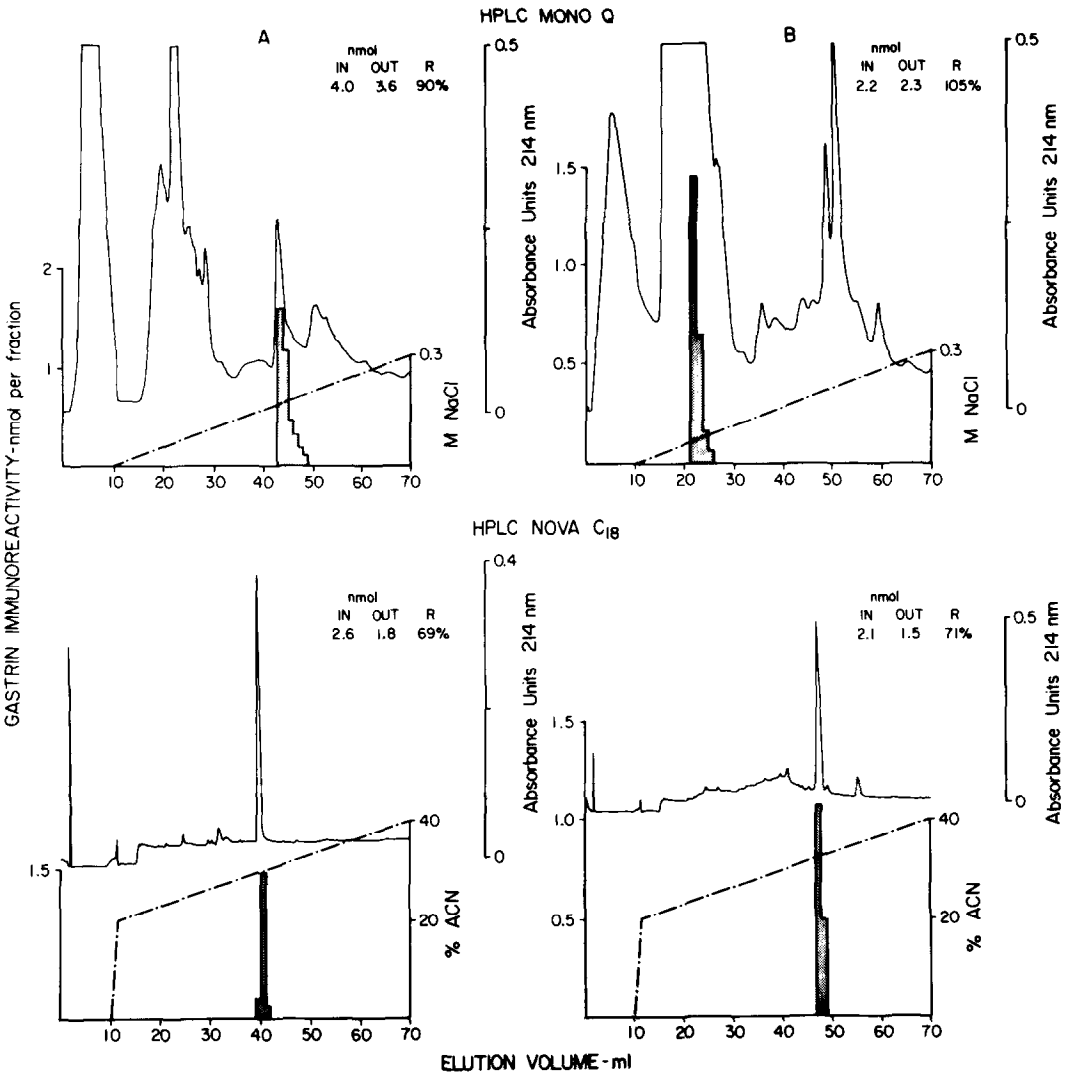


Fig. 2. Subsequent HPLC steps for purification of "little" gastrins, (Peaks A and B from Fig. 1). The Mono Q HR5/5 column was eluted with 0-0.3M NaCl in Tris/20% ACN. The Nova $C_{18}$ cartridge was eluted with 20-40% ACN in 0.1% trifluoroacetic acid.

purification were sufficient to result in very pure products (Fig. 2). Since the "big" gastrins together accounted for only 4.8% of the gastrin-IR, Peaks C and D were not pure after the third HPLC step. However after the PAP-deblocking, the further Nova $C_{18}$ step resulted in a product of sufficient purity for sequencing (Fig. 4).

The sequences of chinchilla "little" and "big" gastrins are compared with the corresponding peptides of the GP and pig in Fig. 5 and the amino acid content following alkaline hydrolysis of purified Peak A is shown in Table I.

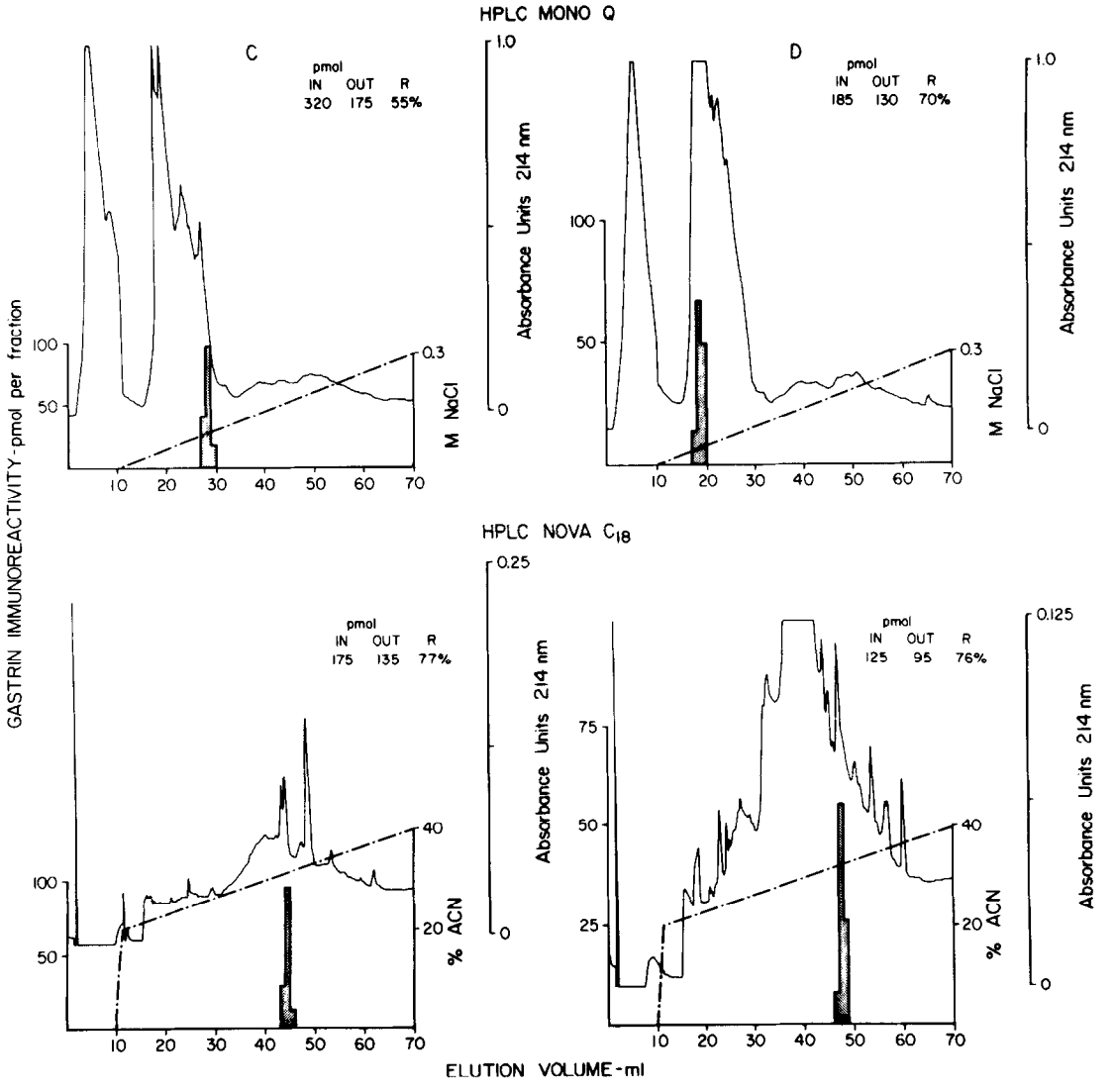


Fig. 3. Subsequent HPLC steps for purification of "big" gastrins, (Peaks C and D from Fig. 1). Elution conditions are the same as in Fig. 2.

There was some ambiguity at position 2 from the $NH_2$-terminus of "big" gastrin. Both Leu and Asp were identified at this position during the sequence analysis. However since Leu is found in this position in the other "big" gastrins that have been purified and sequenced (5,7,9) it is likely that the Asp was a contaminant.

## DISCUSSION

Our hypothesis that the chinchilla gastrins might resemble those of the GP rather than those of Old World mammals appears to be confirmed by the

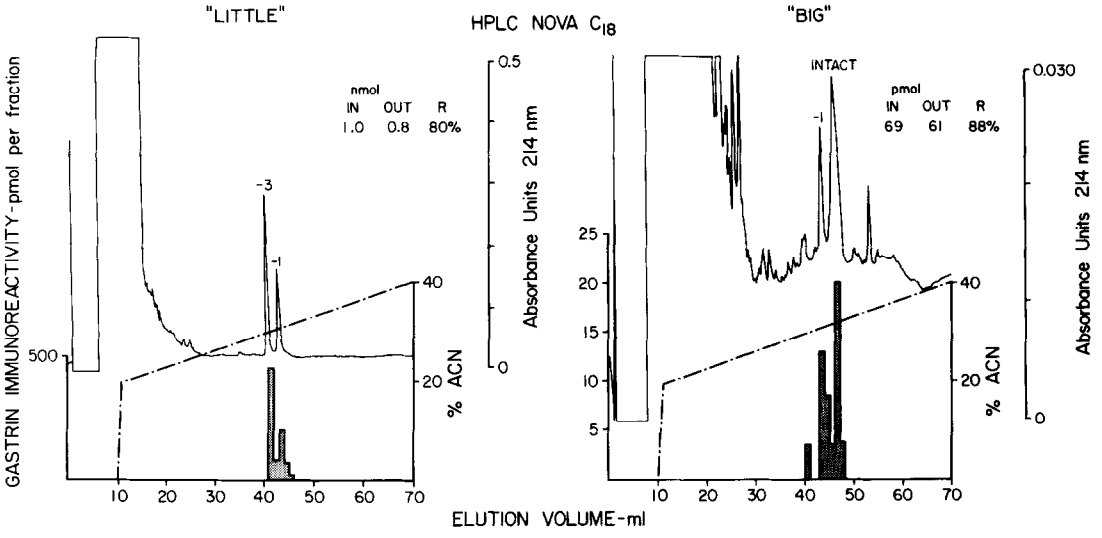


Fig. 4. Final purification step for a portion of "little" (Peak B) and "big" (Peak D) gastrins which were enzymatically deblocked with pyroglutamyl aminopeptidase (PAP). The elution conditions were the same as for the Nova $C_{18}$ cartridge step shown in Fig. 2. Intact represents gastrin which was not deblocked, -1 represents gastrin with pyroglutamic acid removed and -3 represents gastrin with the $NH_2$-terminal tripeptide removed.

present study in that the "little" gastrins of these New World hystricomorphs are identical hexadecapeptides. Our earlier experiences with purification of the "big" gastrins from GP (9), dog and goat (5) made possible the purification and sequencing of chinchilla "big" gastrin even though less than 500 pmol was available for purification. In spite of the fact that the 17 amino acid $NH_2$-terminus of "big" gastrin is not required for its biologic action and has no other known biologic activity, this portion of the molecule is also quite


Fig. 5. Amino acid sequences of chinchilla "little" and "big" gastrins compared with the sequences previously determined for guinea pig and pig. Single letter notation for amino acids is used. <E=pyroglutamic acid and F#=Phe amide.

**TABLE I**

Amino Acid Composition of Chinchilla "Little" Gastrin

| | Peak A | Expected |
|---|---|---|
| D | 0.9 | 1 |
| E | 4.1 | 4 |
| Y($SO_4$) | 1.2 | 1 |
| G | 2.1 | 2 |
| A | 3.2 | 3 |
| M | 0.6 | 1 |
| F | 1.0 | 1 |
| P | ND | 1 |
| W | ND | 2 |

Y($SO_4$) quantitated using alkaline hydrolysate of CCK8(s) as standard.
ND = not determined.

well-conserved. Chinchilla "big" gastrin $NH_2$-terminus differs from the GP peptide in only 4 of the 17 amino acids.

Chinchilla insulin (16) differs from pig insulin in 7 of 51 sites compared to the 17 amino acid difference of GP insulin (17). Since chinchilla insulin resembles the other mammalian insulins more than does GP insulin, it is of particular interest that the "little" gastrins of these two species are identical. This raises the question concerning whether other peptides of the gastroenteropancreatic axis of the New World hystricomorph, the chinchilla, would resemble those of the GP or the corresponding peptides of Old World mammals.

## ACKNOWLEDGMENT

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