Isolation and partial characterization of canine epidermal growth factor/urogastrone

Ryoko KOBAYASHI, Joseph R. REEVE, Jr. and John H. WALSH*

Center for Ulcer Research and Education and UCLA School of Medicine, Veterans Administration,
Wadsworth, Los Angeles, CA 90073, U.S.A.

(Received 18 December 1984/2 April 1985; accepted 10 April 1985)

Canine epidermal growth factor (EGF)/urogastrone was partially purified from dog urine by fractional precipitation with $(\text{NH}_4)_2\text{SO}_4$, ion-exchange chromatography with DEAE-cellulose DE-52, gel filtration with Sephadex G-50, and a second DE-52 chromatography, to yield receptor-competing activity equivalent to $13\text{ }\mu\text{g}$ of standard mouse EGF/litre of starting urine. The purification was monitored by a competitive radioreceptor assay using fixed monolayers of A431 cells. The partially purified canine EGF/urogastrone demonstrated a growth-stimulating activity in 3T3 mouse fibroblast cells as potent as mouse EGF. Analysis by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis revealed one major peptide component with an M_r similar to that of mouse EGF, and two minor peptides of slightly higher M_r . The major peptide component was isolated after reduction and its amino acid composition was determined.

Epidermal growth factor (EGF) is a polypeptide of 53 amino acid residues with three internal disulphide bonds, first isolated by Cohen (1962) from mouse submaxillary glands by its activity to produce precocious eyelid opening in newborn mice. Its marked growth-stimulating effects have been well established both *in vivo* and *in vitro* (Carpenter & Cohen, 1979). The submaxillary glands are found to be an unusually rich source of EGF in mouse, but not in other species. On the other hand, urogastrone was isolated from human urine as a factor that inhibits gastric acid secretion (Gregory & Willshire, 1975) and was subsequently found to be homologous with mouse EGF (Gregory, 1975; Cohen & Carpenter, 1975). In human, EGF-like immunoreactivity was detected in various tissues (Elder *et al.*, 1978; Hirata & Orth, 1979; Oka & Orth, 1983; Elson *et al.*, 1984), but the tissue contents could not account for the amount excreted

daily in the urine (Daily *et al.*, 1978). Although much progress has been made in understanding the effects and the mechanism of interaction of these peptides with target cells in culture (Carpenter & Cohen, 1984), major questions about their physiological role and about the sites of synthesis have yet to be answered. One of the major difficulties in approaching these questions is the fact that the available antibodies to EGF/urogastrone do not cross-react with the homologous peptide from other species (Gregory *et al.*, 1978). In order to establish an animal system in which the physiological effects, the metabolism and the mechanism of action of EGF/urogastrone could be studied, we attempted to isolate the homologous peptide from dog. Previous attempts to measure EGF/urogastrone in dogs by radioimmunoassay have failed, possibly because of immunochemical differences in this peptide between species. It has been known, however, that these peptides bind to the common cell-surface receptors (Cohen & Carpenter, 1975; Hollenberg & Gregory, 1976). Here we describe the partial purification of EGF-like peptide from dog urine by using a convenient radioreceptor assay with fixed monolayers of A431 cells, a human epidermoid-carcinoma-cell line that has an unusually large number of EGF receptors (Fabrizant *et al.*, 1977).

Abbreviations used: EGF, epidermal growth factor; mEGF, mouse EGF, hURO, human urogastrone; rTGF1, rat transforming growth factor type 1; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal-calf serum; phosphate-buffered saline, $0.15\text{ M-NaCl}/0.02\text{ M-sodium phosphate (pH 7.4)}/0.02\% \text{ NaN}_3$; PMSF, phenylmethanesulphonyl fluoride; DTT, dithiothreitol; SDS, sodium dodecyl sulphate.

* To whom requests for reprints should be addressed.

Experimental

Materials

Dog urine was collected daily from several dogs (male and female) by using metabolic cages, filtered through several layers of gauze and stored frozen until use. A431 human epidermoid-carcinoma cells, and 3T3 and NR6 mouse-embryo cell lines were kindly provided by Dr. H. R. Herschman (University of California Los Angeles). The cells were grown at 37°C in DMEM (Morton, 1970) supplemented with 5% (for A431) or 10% (for 3T3 and NR6) FCS (Irvine Scientific, Irvine, CA, U.S.A.) and 50 µg of gentamycin/ml (Irvine Scientific) under humidified atmosphere of CO₂/air (1:10). Gifts of mouse EGF were received from Dr. H. Gregory (ICI, Macclesfield, Cheshire, U.K.) and from Dr. H. R. Herschman, and additional material was prepared by ourselves according to the published method (Savage & Cohen, 1972). Human EGF produced in yeast from a synthetic gene was a gift from Chiron Corp., Emeryville, CA, U.S.A. Na¹²⁵I and [methyl-³H]thymidine were obtained from Amersham International. ¹²⁵I-mEGF was prepared by the chloramine-T method (Moriarty & Savage, 1980) to a specific radioactivity of (1.7–2.7 × 10⁵ c.p.m./ng. *M_r* marker proteins were purchased from Bio-Rad. DEAE-cellulose (DE-52) was purchased from Whatman and Sephadex from Sigma. Sep-Pak C₁₈ cartridges were from Waters. Constant-boiling HCl for amino acid analysis was obtained from Pierce Chemical Co. Other chemicals used were of reagent grade.

Radioreceptor assay with fixed A431 cells

A431 cells were plated at 10⁴ cells/0.2 ml per well in 96-well Costar plates and were grown to confluency. The plates were washed three times with ice-cold phosphate-buffered saline, immersed for 5 min in ice-cold 0.25% glutaraldehyde in phosphate-buffered saline, then washed four times with ice-cold phosphate-buffered saline. The fixed plates were filled with 0.5% bovine serum albumin/0.1 M-glycine/0.05% NaN₃ in phosphate-buffered saline and left at room temperature at least for 1 h, then stored at 4°C until use. The fixation and storage conditions were adapted from the method described by Heusser *et al.* (1981). For the assay, washed cells were exposed (0.1 ml/well) to (1–2) × 10⁵ c.p.m. of ¹²⁵I-mEGF with various amounts of unlabelled mEGF (for calibration standards) or with samples to be tested in the binding medium, which was 0.2% bovine serum albumin/0.8% NaCl/0.02% KCl/0.02% KH₂PO₄/0.15% Na₂HPO₄ (pH 7.2)/0.01% NaN₃, and incubated at ambient temperature for 2–6 h. After the plates had been washed four times with

ice-cold phosphate-buffered saline, the cell-associated radioactivity was measured after solubilizing cells with 0.5 M-NaOH (0.1 ml/well), by absorbing the well content to cotton swabs and counting them in tubes in a γ-radiation counter (Tracor Analytic). The inhibitory activity of the samples to the receptor binding of ¹²⁵I-mEGF was expressed as mEGF equiv., that is, the amount of standard mEGF with equivalent inhibitory activity.

Assay for DNA synthesis

The confluent monolayers of 3T3 and NR6 cells in 24-well Costar plates, grown in 5% FCS-containing DMEM, were treated with test reagent solutions (50 µl/ml of culture medium) for 24 h, then were pulsed for 2 h with [methyl-³H]thymidine (0.5 µCi/ml). The cells were washed with ice-cold phosphate-buffered saline, then treated with cold 10% trichloroacetic acid (1 ml/well) for 15 min. The trichloroacetic acid-precipitable material, after washing with cold phosphate-buffered saline, was solubilized with 0.5 M-NaOH (0.5 ml/well) for 2 h then 0.4 ml of each well content was transferred into a scintillation vial containing 0.4 ml of 0.5 M-HCl, mixed with 10 ml of ACS Scintillant (Amersham) and the radioactivity counted in a liquid-scintillation counter (Nuclear Chicago model 6868).

Purification of canine urogastrone

All procedures were carried out at 4°C.

Step 1. The frozen urine (11 litres, total A₂₈₀ 594000) was thawed and PMSF was added to a final concentration of 0.1 mM. Solid (NH₄)₂SO₄ was added to 30% saturation and, after several hours of stirring, the precipitates (ppt._{0–30}) were separated by centrifugation at 4500 g for 30 min. To the supernatant (NH₄)₂SO₄ was added to 60% saturation and the precipitates (ppt._{30–60}) collected after overnight stirring. The precipitates at 80% saturation of the salts (ppt._{60–80}) were collected in a similar manner. The precipitates were separately extracted with 1 mM-PMSF (30–100 ml), and EGF-like activity in each extract was determined after dialysis against 0.15 M-NaCl/0.01 M-sodium phosphate, pH 7.4, by the radioreceptor assay with serially diluted samples.

Step 2. The extract (100 ml) from ppt._{30–60}, where most of total precipitable activity was found, was dialysed against 0.01 M-Tris/HCl, pH 8.2 containing 0.2 mM-PMSF and chromatographed on a DE-52 column (5 cm × 27 cm) at a flow rate of 36 ml/h. The column was eluted with a linear gradient of 0–0.3 M-NaCl in the same buffer (total 3.8 litres), followed by elution with 1 M-NaCl in the same buffer and finally with 1 M-NaCl/0.5 M-NaOH. The active fractions were combined,

dialysed against distilled water (in Spectrapor 3; M_r cut-off 3500) and freeze-dried.

Step 3. The freeze-dried powder (a half portion; 110 μ g of mEGF equiv. activity) was extracted with 0.3M-NaCl/0.02M-sodium phosphate (pH 7.4)/0.02% NaN_3 (total A_{280} 156) and subjected to gel filtration over a column (2.5 cm $\times$ 93 cm) of Sephadex G-50 (fine grade) in the same solution at a flow rate of 20 ml/h. The fractions with a minor earlier-to-be-eluted peak (14% of the total activity, fraction I) and a major peak (86% of the total activity, fraction II) were collected.

Step 4. Fraction II (total A_{280} 12.7) was desalted by allowing it to adsorb to a Sep-Pak C_{18} cartridge and by eluting with acetonitrile/water (1:1, v/v) containing 0.1% trifluoroacetic acid. After the eluate had been dried *in vacuo* it was dissolved in 0.02M-ammonium acetate, pH 5.6, then chromatographed on a second DE-52 column (0.9 cm $\times$ 90 cm) with a linear gradient (0.02–0.2M) of the same buffer (total 180 ml), followed by a second gradient (0.2–0.7M), again of the same buffer (total 120 ml). The column was further washed with the same buffer at 1 M and finally with 20% (v/v) acetic acid/1 M-ammonium acetate (flow rate 12 ml/h). Two fractions with EGF-like activity were collected; fraction IIa (activity 15 μ g of mEGF equiv., total A_{280} 0.36) eluted at 0.02M-ammonium acetate and fraction IIb (activity 69 μ g of mEGF equiv., total A_{280} 1.23), eluted at 0.11M-buffer. Fractions IIa and IIb were analysed for amino acid compositions after desalting (results not shown).

Isolation of the 6 kDa peptide(s) from partially purified canine urogastrone

A half portion of fraction IIb was treated with 0.05M-DTT/6M-guanidinium chloride for 24 h at room temperature then at 40°C for 30 min. The mixture (0.2 ml) was fractionated on a column (0.6 cm $\times$ 60 cm) of Sephadex G-50 (superfine grade) in 2M-DTT/6M-guanidinium chloride at room temperature and A_{280} was monitored. The first peak (fraction 1) was eluted at 8.5 ml (46% of the total column volume), close to the elution position of a reduced sample of mEGF (results not shown). A second peak was eluted at 12.2 ml. These two peak fractions were collected and desalted. They were analysed by SDS/polyacrylamide-gel electrophoresis and for amino acid compositions.

Other methods

SDS/polyacrylamide-gel electrophoresis was performed with 0.5 mm-thick microslab gel of 20% (w/v) acrylamide, by a method based on that described by Maizel (1971). Amino acid analysis

was performed in an amino acid analyser (System 6300, Beckman Instruments) equipped with a recording integrator (3390A, Hewlett-Packard). The samples (1–5 μ g) were hydrolysed with 0.2 ml of constant-boiling HCl in evacuated sealed tubes for 24 h at 110°C. Tryptophan was analysed by the method of Matsubara & Sasaki (1969). Performic acid oxidation was carried out in a separate tube for each sample to be analysed, by a method based on that described by Hirs (1967).

Results and discussion

Assay system

Although the available antibodies to mouse EGF or to human urogastrone show little cross-reactivity between species, it has been demonstrated in cultured cells and membrane preparations that the two peptides interact with common receptors with comparable affinities. A competitive radioreceptor assay with A431 cells proved to be useful to detect EGF-like activity in dog urine and in the purification. Use of the fixed monolayers of the cells simplified the routine assay and gave reproducible results. Fig. 1 compares the standard inhibition curves with mEGF for ^{125}I -mEGF binding assayed with cells that had been stored for the indicated periods after fixation. The half-point of the curves fell at 2–4 ng (0.3–0.7 pmol) of mEGF. The fixed cells could be used for at least 3 months after fixation without appreciable deterioration. Quantification of human urogastrone preparation by this method was also successful (results not shown).

Fig. 1. Competitive radioreceptor assay of EGF/urogastrone

Inhibition of ^{125}I -mEGF binding to the fixed monolayers of A431 cells by standard mEGF shown as a function of mEGF concentration. The cells were stored for the following periods after fixation: $\square$, 3 days; $\circ$, 2 weeks; $\bullet$, 6 weeks; $\triangle$, 10 weeks.

Purification of canine urogastrone

We could detect EGF-like activity in dog urine samples, but quantification was difficult because the inhibition was not parallel with the standard curve, possibly because of interfering components in the urine. Thus, in the following experiment (Scheme 1), the concentration of the EGF-like activity in the starting, combined, urine was estimated as 41 and 95 μg of mEGF equiv./litre from 1:8- and 1:4-diluted urine respectively. Therefore a tentative estimation of EGF/urogastrone was attempted by assaying serially diluted samples and by averaging the values from the assay points closer to the half-maximum of the standard inhibition curve in order to calculate the recoveries in the purification steps (Table 1). By fractional $(\text{NH}_4)_2\text{SO}_4$ precipitation, a major EGF-like ac-

tivity was detected in the extract of ppt.₃₀₋₆₀. Subsequent DE-52 chromatography at pH 8.2 (Fig. 2) removed about 90% of the u.v.-absorbing material associated with the active component(s) in the extract and the quantification improved. A total activity of 310–400 μg of mEGF equiv. was detected in the pooled active fractions at dilution from 1:50 to 1:200. When a half portion of this pool was subjected to gel filtration (Fig. 3), a major activity peak (fraction II, 110 μg mEGF equiv.) was eluted at a similar elution volume to that of human urogastrone, whose M_r is about 6200 (Gregory, 1975), and a minor peak that was eluted at a higher- M_r region was noted and appeared to increase after sample storage, suggesting aggregate formation. Further purification of fraction II by a second DE-52 chromatography at pH 5.6 with a buffer system based on a method used for

Scheme 1. Purification of canine urogastrone

Experiments were performed at 4°C throughout, except the last step, to obtain fraction A. See the Experimental section for details.

Table 1. Purification of canine EGF/urogastrone

In this preparation 11 litres of dog urine were used, but only a part of the sample was processed after the Sephadex G-50 step. See the Experimental section for details of each step. The activity was measured as mEGF equiv. by the competitive radioreceptor assay. The degree of purification is expressed by the ratio of the activity to the A_{280} .

Fraction	Total A_{280} units	Activity/litre of urine (μg of mEGF equiv.)	Recovery of activity (%)	Purification (fold)
Raw urine	594000	68	100	1
$(\text{NH}_4)_2\text{SO}_4$ ppt. ₃₀₋₆₀	2630	57	84	190
DE-52 (1st) active pool	300	32	47	940
Sephadex G-50 fraction II	25	27	40	9300
DE-52 (2nd) fraction IIb	2.5	13	19	45000

Fig. 2. DE-52 chromatography of the extract of ppt.₃₀₋₆₀ (step 2)

The extract of $(\text{NH}_4)_2\text{SO}_4$ precipitate, ppt.₃₀₋₆₀ (total A_{280} 2626 after dialysis against 0.01 M-Tris/HCl, pH 8.2), was applied to a DE-52 column (5 cm $\times$ 27 cm) equilibrated with the same buffer, and was eluted with a NaCl gradient from 0 to 0.3 M (----) in the same buffer at a flow rate of 36 ml/h. EGF-like activity in the fractions was determined by the radioreceptor assay (●) and the active fractions (tubes 223-259) were combined.

Fig. 3. Gel filtration of the active fraction from the first DE-52 column (step 3)

A 6 ml sample (total A_{280} 156) was chromatographed on a column (2.5 cm $\times$ 93 cm) of Sephadex G-50 (fine grade) in 0.3 M-NaCl/0.02 M-sodium phosphate (pH 7.4)/0.02% NaN_3 at a flow rate of 16.4 ml/h. The active fractions were pooled as fractions I and II. V_0 and V_t represent void volume and total column volume respectively.

purification of mEGF (Savage & Cohen, 1972) and human urogastrone (Savage & Harper, 1981) gave a major activity peak (fraction IIb, 69 μg of mEGF equiv.) that was eluted at 0.11 M-ammonium acetate, and another (fraction IIa, 15 μg mEGF equiv.) that was eluted with the first buffer (Fig. 4).

Overall recovery of the EGF-like activity in fraction IIb (which will be referred to as 'partially purified canine urogastrone'), on the basis of activity detected from the first DE-52 column, was about 40% and corresponded to 13 μg of mEGF equiv./litre of urine. This value, estimated by the

Fig. 4. DE-52 chromatography of fraction II at pH 5.6 (step 4)

The sample (total A_{280} 12.7 after desalting) was chromatographed on a DE-52 column (0.9 cm $\times$ 9 cm) equilibrated with 0.02 M-ammonium acetate, pH 5.6, and eluted with two sets of gradients (0.02–0.2 M and 0.2–0.7 M) of the same buffer (----). Active fractions Ila (tubes 21–25) and I Ib (tubes 102–118) were pooled respectively.

radioreceptor assay, with an mEGF preparation (from Dr. H. Gregory) as standard, was approx. 60% of the actual protein content of fraction I Ib calculated from the amino acid analysis.

Properties of the partially purified canine urogastrone

Fraction I Ib stimulated DNA synthesis in quiescent mouse fibroblast cells (3T3) in culture with a potency similar to that of mouse EGF (Fig. 5). The protein concentration used here was based on the amino acid analysis. As expected, neither mEGF nor canine urogastrone stimulated DNA synthesis in NR6 cells, a mutant cell line derived from 3T3 cells that lack functional EGF receptors (Pruss & Herschman, 1977). Both 3T3 and NR6 cells responded when FCS was added at 10% (v/v) to the medium. The results suggest that the material tested in this assay exerted the growth-stimulating effect through binding to the EGF receptor.

Analysis by SDS/polyacrylamide-gel electrophoresis indicated that fraction I Ib was a mixture of a major peptide(s) with an M_r similar to that of reduced mEGF (M_r approx. 6000) and at least two larger peptides (Fig. 6). [A major portion of our mEGF sample migrated as an aggregated form (possibly a dimer) under the experimental conditions used. Such abnormal behaviour of mEGF on SDS/polyacrylamide-gel electrophoresis has often been observed by other investigators (H. R. Herschman, personal communication; Bridgen, 1981) and by one of us (R. Kobayashi, unpublished work). The experiment with reducing reagent showed only partial dissociation of mEGF into a monomer form; owing to insufficient amount of the reagent (Fig. 6).] The two bands with mobilities slightly lower than that of the 6 kDa peptide band on the gel disappeared after reduction, leaving a

Fig. 5. Enhancement of DNA synthesis in 3T3 cells by canine urogastrone fraction I Ib

Confluent monolayers of 3T3 and NR6 cells were incubated for 24 h in the culture medium with various concentrations of fraction I Ib ($\circ$, $\bullet$), as well as 20 and 40 ng/ml of mEGF ($\square$, $\blacksquare$) as positive (for 3T3) and negative (for NR6) controls, and 10% FCS ($\triangle$, $\blacktriangle$) as positive controls for both types of cells. After a 2 h pulse with [3 H]thymidine, trichloroacetic acid-precipitable radioactivity was determined. The canine urogastrone concentration was based on amino acid analysis. Open symbols represent 3T3 cells and closed symbols, NR6 cells.

major EGF-like band and a minor band moving to the buffer front. Because of the apparently increased yield after reduction, we attempted isolation of the 6 kDa peptide(s) by gel filtration

Fig. 6. SDS/polyacrylamide-gel electrophoresis of EGF/urogastrone

The samples with or without reducing agent (2-mercaptoethanol) were run on a 20%-(w/v)-acrylamide microslab gel, which was stained with Coomassie Blue to detect peptides. The samples shown are, from left to right; human urogastrone (hURO; produced in yeast from synthetic gene; gift from Chiron Corp.), mouse EGF (mEGF) and canine urogastrone fraction IIb (cURO) (all without 2-mercaptoethanol); mouse EGF (mEGF), canine urogastrone fraction IIb (cURO) and M_r marker proteins (Std.) (all with 2-mercaptoethanol). The M_r markers used were: phosphorylase *b* (92500), bovine serum albumin (66200), ovalbumin (45000), carbonic anhydrase (31000), soya-bean trypsin inhibitor (21500) and lysozyme (14400), 'BPB' indicates the Bromophenol Blue dye front.

after reduction under denaturing conditions (see the Experimental section for details). The major peptide(s) were eluted between 41 and 52% of the total column volume (Fig. 7, fraction A), similarly to reduced mEGF (results not shown). By amino acid analysis, fraction A, after being desalted, accounted for 50% of the protein content of the starting material (fraction IIb). The first half of the second peak (Fig. 8, fraction B) contained only the Coomassie Blue-positive component(s) which moved to the buffer front on the SDS/polyacrylamide gel, and the second part (fraction C) with no detectable band on the gel. The last, high-u.v.-absorbing, peak at the salt position also was detected in the chromatogram of reduced mEGF, and it probably represents the reducing reagent. The fractions B, C and D were not characterized further, and the identities of the u.v.-absorbing components in these fractions are not known.

Fig. 7. Gel filtration of a reduced sample of the partially purified canine urogastrone

A sample of fraction IIb was treated with 0.005M-DTT/6M-guanidinium chloride (200 μ l), then fractionated on a column (0.6 cm $\times$ 60 cm) of Sephadex G-50 (superfine grade) in 2mM-DTT/6M-guanidinium chloride HCl at room temperature at a flow rate of 3 ml/h. Fractions were pooled (A, B, C and D) as indicated by the horizontal bars.

The amino acid composition of the isolated major peptide(s) (fraction A) is shown in Table 2. Assuming an M_r of approx. 6000, the number of residues per molecule of the peptide was tentatively assigned for comparison with those reported for human urogastrone and mouse EGF. The compositions are similar in their high content of aspartic acid/asparagine, glutamic acid/glutamine, glycine and cysteine, the presence of methionine, tryptophan and two isoleucine residues, whereas the presence of two phenylalanine residues and the relatively low content of tyrosine in canine fraction A contrasted with the other two peptides. Only 0.5 tryptophan residue/molecule was recovered by hydrolysis with 2% thioglycolic acid-containing HCl and 4.1 residues of cysteic acid/molecule from the performic acid-oxidized samples, compared with 1.1 tryptophan residue and 5.7 cysteic acid residues from mEGF in parallel experiments. Ambiguity about the composition will be clarified only when the complete amino-acid-sequence data become available.

The recent work on rat TGF type 1 (Marquardt *et al.*, 1984) has brought a new aspect to the study of EGF/urogastrone-type peptides. It was found to be a 50-residue peptide structurally and functionally homologous with EGF, but lacking certain amino acids such as a methionine residue at position 21 and two tryptophan residues at positions 49 and 50, positions that had been thought to be invariant in peptides of this family. The data suggest that these residues are not indispensable for binding to the EGF receptor or in the growth-stimulating activity. The amino acid composition of rTGF1 is included in Table 2.

Table 2. *Amino acid composition of canine urogastrone peptide fraction A and other EGF-like peptides*

The results are given without correction as average values from the analysis of duplicate samples after 24h hydrolysis at 110°C with constant-boiling HCl in evacuated sealed tubes. The content of cysteine was determined as cysteic acid after performic acid oxidation. Tryptophan was determined by the method of Matsubara & Sasaki (1969). 'cURO' in the Table represents canine EGF/urogastrone peptide fraction A.

		Composition (residues/molecule)					
Amino acid	Peptide . . .	Present work			Literature values (nearest integer)		
		cURO	cURO†	mEGF	mEGF‡	hURO§	rTGF
Asx		5.8	6	6.8	7	7	3
Thr		2.8	3	1.7	2	0	2
Ser		3.3	4	4.0	6	3	3
Glx		6.3	6	3.1	3	5	5
Pro		3.7	4	1.5	2	1	2
Gly		4.9	5	5.6	6	4	3
Ala		1.9	2	0.2	0	2	3
Val		3.9	4	1.9	2	3	6
Met		0.4	1	0.6	1	1	0
Ile		1.9	2	1.9	2	2	0
Leu		3.1	3	3.8	4	5	3
Tyr		1.3	1(2)	4.4	5	5	2
Phe		1.9	2	0.0	0	0	3
His		1.1	1	0.9	1	2	5
Lys		2.1	2	0.0	0	2	2
Arg		2.1	2	3.8	4	3	2
Cys		4.1	4(6)*	5.7	6	6	6
Trp		0.5	1	1.1	2	2	0
Total no. of residues . . .			(52–55)		53	53	50

* The possibility of six residues per molecule in cURO fraction A remains, since the same amounts (3.7 residues/molecule) of cysteine were recovered from unoxidized samples of both cURO and mEGF.

† To nearest integer, assuming an M_r of ~6000.

‡ From Savage *et al.* (1972).

§ From Gregory (1975).

|| From Marquardt *et al.* (1984).

Comparison of the compositional values illustrates that even structurally and functionally homologous peptides can have amino acid compositions quite different from each other. The lack of apparent similarity except for cysteine helps to explain their immunochemical differences.

At present it is not known whether those peptides with a higher M_r than that of mEGF in our partially purified canine urogastrone sample represent native forms or artefacts generated during purification. Their ability to inhibit gastric acid secretion could not be tested because of the limited amount of the material available. Further purification of the active canine urogastrone and improvement of the yield (to permit the sequence study and to raise specific antibodies) are required.

This study was supported by National Institutes of Health grant AM17328 and by the Veterans Administrations. We thank Dr. H. R. Herschman for making his cell-culture facilities available to us to perform all the cell-culture experiments reported in this paper, and also for his gifts of mEGF and the A431, 3T3 and NR6 cells.

We also thank Dr. H. Gregory for generously providing human urogastrone and mEGF, and Chiron Corp. for the yeast-produced human urogastrone.

References

- Bridgen, P. (1981) *Biologue*, pp. 1–3, BRL Publications, Bethesda Research Laboratories, Gaithsburg, MD
- Carpenter, G. & Cohen, S. (1979) *Annu. Rev. Biochem.* **48**, 193–216
- Carpenter, G. & Cohen, S. (1984) *Trends Biochem. Sci.* **9**, 169–171
- Cohen, S. (1962) *J. Biol. Chem.* **237**, 1555–1562
- Cohen, S. & Carpenter, G. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1317–1321
- Daily, G. E., Kraus, J. W. & Orth, D. N. (1978) *J. Clin. Endocrinol. Metab.* **46**, 929–936
- Elder, J. B., Williams, G., Lacey, E. & Gregory, H. (1978) *Nature (London)* **271**, 466–467
- Elson, S. D., Browne, C. A., & Thorburn, G. D. (1984) *J. Clin. Endocrinol. Metab.* **58**, 589–594

- Fabricant, R. N., Delarco, J. E. & Todaro, G. J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 565-569
- Gregory, H. (1975) *Nature (London)* **257**, 325-327
- Gregory, H. & Willshire, I. R. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 1765-1774
- Gregory, H., Holmes, J. E. & Willshire, I. R. (1978) in *Methods in Hormone Radioimmunoassay*, 2nd edn. (B. N. Jaffe & H. R. Behrman, eds.), pp. 927-939, Academic Press, New York
- Heusser, C. H., Stocker, J. W. & Gisler, R. H. (1981) *Methods Enzymol.* **73**, 406-418
- Hirata, Y. & Orth, D. N. (1979) *J. Clin. Endocrinol. Metab.* **48**, 667-672
- Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 59-62
- Hollenberg, M. D. & Gregory, H. (1976) *Life Sci.* **20**, 267-274
- Maizel, J. V. (1971) *Methods Virol.* **5**, 179-246
- Marquardt, H., Hunkapiller, M. W., Hood, L. E. & Todaro, G. J. (1984) *Science* **223**, 1079-1082
- Matsubara, H. & Sasaki, R. M. (1969) *Biochem. Biophys. Res. Commun.* **35**, 175-181
- Moriarity, D. M. & Savage, C. R., Jr. (1980) *Arch. Biochem. Biophys.* **203**, 506-518
- Morton, H. J. (1970) *In Vitro* **6**, 89-108
- Oka, Y. & Orth, D. N. (1983) *J. Clin. Invest.* **72**, 249-259
- Pruss, R. M. & Herschman, H. R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3918-3921
- Savage, C. R., Jr. & Cohen, S. (1972) *J. Biol. Chem.* **247**, 7609-7611
- Savage, C. R., Jr. & Harper, R. (1981) *Anal. Biochem.* **111**, 195-202
- Savage, C. R., Jr., Inagami, T. & Cohen, S. (1972) *J. Biol. Chem.* **247**, 7612-7621