Gut, 1978, **19**, 655-663

Gastrointestinal somatostatin: extraction and radioimmunoassay in different species

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SUMMARY A radioimmunoassay capable of detecting 300 fg somatostatin has been developed and levels of the polypeptide in gastrointestinal tissues from man, dog, and rat have been measured. Rapid freezing of collected samples and careful control of extraction is necessary. Concentrations in different regions of dog antrum (425 ± 50 to 773 ± 254 ng/g tissue) are similar to those in antrum from duodenal ulcer patients and control subjects: 614 ± 125 and 465 ± 104 ng/g tissue respectively. Levels in histologically normal human pancreas (253 ± 43 ng/g tissue) are comparable with those in dog pancreas (333 ± 66 ng/g tissue), whereas in two cases of neonatal hypoglycaemia the concentration exceeded 3000 ng/g tissue. On gel chromatography the majority of immunoreactive somatostatin elutes as the synthetic tetradecapeptide and a small fraction as a larger species.

Somatostatin exerts potent inhibitory action on the release of a number of gastrointestinal and pancreatic hormones. The *in vivo* secretion of insulin (Alberti *et al.*, 1973; Koerker *et al.*, 1974; Ward *et al.*, 1975), glucagon (Dobbs *et al.*, 1975), gastrin (Bloom *et al.*, 1974; Arnold *et al.*, 1975), secretin (Boden *et al.*, 1975), gastric inhibitory polypeptide (Pederson *et al.*, 1975) and motilin (Bloom *et al.*, 1975) are suppressed under normal or pathological conditions by somatostatin infusion. Inhibition of insulin (Efendic *et al.*, 1974; Okamoto *et al.*, 1975), glucagon (Iversen, 1974), and gastrin (Hayes *et al.*, 1975) release has been demonstrated with *in vitro* systems. In addition, somatostatin infusion causes direct inhibition of gastric secretion (Bloom *et al.*, 1974; Arnold *et al.*, 1975; Arnold and Creutzfeldt, 1975), pancreatic juice and enzyme secretion and gall bladder contraction (Creutzfeldt *et al.*, 1975; Lankisch *et al.*, 1975).

Apart from the brain, somatostatin has been shown by immunohistochemistry to occur in the pancreas, stomach, duodenum, and jejunum (Luft *et al.*, 1974; Goldsmith *et al.*, 1975; Dubois, 1975; Polak *et al.*, 1975; Rufener *et al.*, 1975), and specific localisation to the D-cell has been reported (Polak *et al.*, 1975; Rufener *et al.*, 1975). Direct measurement of somatostatin in rat and chicken tissues by radioimmunoassay (Arimura *et al.*, 1975a,b; Weir *et al.*, 1976; Kronheim *et al.*, 1976; Vale *et al.*, 1976) has confirmed the gastrointestinal localisation. Release of immunoreactive somatostatin from isolated pancreatic islets incubated *in vitro*, and from the perfused pancreas, occurs in the presence of a number of physiological stimuli (Schauder *et al.*, 1976, 1977a,b; Barden *et al.*, 1976; Patton *et al.*, 1976a,b), suggesting a role for somatostatin as a regulator of pancreatic secretion. The present communication examines the importance of collection and extraction procedures for measurement of somatostatin in the gastrointestinal tract and the levels of the polypeptide in these tissues from man, dog, and rat.

Methods

IMMUNISATION

One milligram of synthetic cyclic somatostatin (lot no. 4999, Serono, Freiburg (Br) Germany) was dissolved in 200 µl distilled water. To the peptide was added 8 mg bovine serum albumin (Behringwerke, Marburg/Lahn, Germany) in 1 ml water and, dropwise, 50 mg 1-ethyl 3(3-dimethylamino-propyl)-carbodiimide HCl (Sigma) dissolved in 500 µl distilled water. The reaction mixture was shaken gently, left at room temperature for four hours and the suspension dialysed for 48 hours against distilled water at 4°C. When ^{125}I-somatostatin was added to the coupling mixture 71·6 ± 3·4% (eight coup-

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Received for publication 5 January 1978

lings) of the radioactivity was retained during dialysis. The mass of lyophilised conjugate was 9·5 ± 0·1 mg (eight couplings). Molar coupling ratios were therefore between 3·6 and 5·0 mol somatostatin per mol albumin.

The conjugate was emulsified in an equal volume of Freund's complete adjuvant and injected intradermally in the neck of New Zealand white rabbits in a dose of 250 μg per animal. Immunisation was repeated three times at monthly intervals and 10 days after each immunisation blood was taken from an ear vein for testing antibody reactivity by radioimmunoassay.

IODINATION OF SOMATOSTATIN AND PURIFICATION OF THE LABELLED PEPTIDE

Iodination of 1-Tyr-somatostatin was performed by a slight modification of the method of Greenwood *et al.* (1963). In a reaction volume of 30 μl, 1 mCi ^{125}I was reacted with 5 μg 1-Tyr-somatostatin (Serono, Freiburg, Germany) by the addition of 20 μg chloramine-T. After a 15 second incubation the reaction was terminated by the addition of 50 μg sodium metabisulphite. The labelled peptide was separated from free ^{125}I and 'damaged' material by adding hormone free plasma (1 ml) followed by 20 mg QUSO G32 (Philadelphia Quartz Co., USA). After centrifugation the pellet was washed twice with 1 ml distilled water, the labelled somatostatin eluted with acetic acid/acetone/water (0·1:3·9:4·0), diluted with 0·1 M acetic acid containing 0·05% human serum albumin, and lyophilised. A mean of 59 ± 3% (nine iodinations) of initial radioactivity was eluted from the QUSO G32. Before use in the assay the ^{125}I-somatostatin was purified on a CM-cellulose column (Whatman CM 52) essentially by the method of Arimura *et al.* (1975a). The lyophilised material (1-3 × 10^6 cpm) was dissolved in 1 ml 0·002 M ammonium acetate (pH 4·6) and applied to the CM-cellulose equilibrated in the same buffer. After washing the column with 20 ml 0·002 M ammonium acetate the assay tracer was eluted with 0·2 M ammonium acetate (pH 4·6) (Fig. 1).

Approximately 90% of added counts appeared in the major peak with freshly labelled material. Fractions from the top of the peak and those from the descending limb, amounting to 40-45% of the peak radioactivity, gave equally good assays and had specific activities of 900-1100 μCi/μg. The overall elution was reproducible over a three month period, although the first peak demonstrated a significant increase.

The purification of ^{125}I-somatostatin by such ion-exchange chromatography resulted in a marked improvement in the assay: with freshly labelled material the unspecific binding decreased from 10% to 2·5% and a five-fold increase in assay sensitivity was achieved.

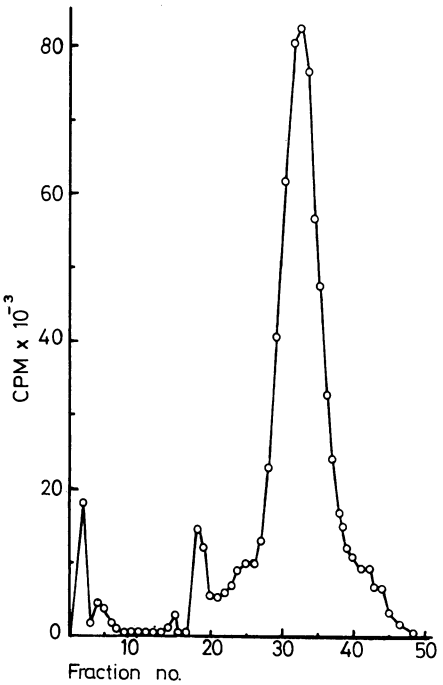

Fig. 1 *Purification of ^{125}I-somatostatin on CM-cellulose. ^{125}I-somatostatin was applied to a CM-cellulose (CM 52) column (5 × 1 cm) in 0·002 M ammonium acetate pH 4·6 and the column washed with 24 ml of the same buffer. Purified tracer was eluted with 0·2 M ammonium acetate pH 4·6 and fractions 31-33 pooled for the assay.*

CONDITION OF ASSAY

Incubations were carried out in triplicate in glass tubes for 48 hours at 4°C. The total incubation volume of 400 μl consisted of 100 μl antiserum, 100 μl synthetic cyclic somatostatin standard or unknown, 100 μl ^{125}I-Tyr somatostatin (0·5 f mol), and 100 μl diluent buffer. Diluent buffer consisted of 50 mM barbital buffer (pH 7·4) containing merthiolate (0·01%), 500 KIU/ml aprotinin (Trasylol, Bayer), and 1% human serum albumin. Separation of bound and free antigen was achieved by the dextran coated charcoal method; 2·5 g charcoal (Norit A) and 0·5 g Dextran T70 (Pharmacia, Sweden) were mixed in 200 ml 0·05 M phosphate buffer (pH 7·4) and stirred for at least 30 minutes at 4°C. One millilitre of the mixture was added to each tube and after vortex mixing the tubes were centrifuged at 3000 rpm for 15 minutes at 4°C. After the supernatant was decanted both bound and

free fractions were counted in an automatic γ-spectrometer.

TISSUE COLLECTION AND EXTRACTION

Tissues from dogs were removed under anaesthesia and kept under a variety of conditions (as described in the text) before storage at $-20°C$. Rat tissues were removed after cervical dislocation and snap frozen on dry ice. All animals had starved for at least 12 hours before removal of tissue and, to ensure that the rats had completely empty stomachs, the period of starvation was preceded by three days on a liquid formula diet (Vivasorb, Pfrimmer, Erlangen, Germany).

Tissue sampling during endoscopy was performed after an overnight fast and after intravenous injection of 10 mg diazepam immediately before the start of gastroscopy. Surgical biopsies were obtained during operations for duodenal ulcer. Antrum mucosa was excised 1-3 cm proximal to the pylorus. Corpus mucosa was obtained from the greater curvature opposite to the angle of the stomach. Resected pancreas was collected as soon as possible after the operative procedure. Inevitable delays between operation and subsequent freezing, and the relative heterogeneity of material, may partly contribute to the wide interpatient variation as discussed in the text.

Control stomach samples were from subjects with normal basal and pentagastrin stimulated gastric secretion and consisted of normal volunteers, patients with dyspeptic complaints, or patients undergoing duodenopancreatectomy as a result of chronic pancreatitis. Histologically normal pancreatic specimens were from clinically normal accident cases (two) or patients with Verner-Morrison syndrome (one), Zollinger-Ellison syndrome (one), or insulinoma (11).

Unless otherwise stated, for extraction the frozen tissue was quickly weighed, boiled for 15 minutes in 0·2 M acetic acid (10 mg/ml) and homogenised with an Ultratorax homogeniser. Solid material was removed by centrifugation (10 000 *g* 10 minutes) and the clear supernatant lyophilised. For assay, the extract was dissolved in distilled water and suitably diluted with diluent buffer. All extracts were measured in a minimum of three dilutions and when results differed by more than 10% the sample was reassayed.

GEL-FILTRATION CHROMATOGRAPHY

Chromatography was performed on columns of Sephadex G25 fine (100 × 1 cm). Elution was with 0·2 M acetic acid at a flow rate of 5 ml/h. Collected fractions (1 ml) were lyophilised and dissolved in assay buffer. Void volume (Vo) and total volume (Vt) were determined using blue dextran and ^{125}I respectively. SOM indicates the elution position of synthetic cyclic somatostatin.

EXPRESSION OF RESULTS

All data presented are in the form mean ± SEM with the number of experiments or measurements in brackets (n).

Results

ASSAY

Cyclic somatostatin conjugated to bovine serum albumin was relatively immunogenic and produced antibodies in all four rabbits immunised after two immunisations, which bound 50% of tracer at titres ranging from 1:200 to 1:8000. Assays with these antibodies were capable of detecting 2 pg cyclic somatostatin per assay tube. In current assays using an antibody after three immunisations (No. VI/3/1) at a dilution of 1:12 500 concentrations as low as 300 femtogram can be detected with 95% confidence (Fig. 2). No cross-reaction occurred with a number of other hormones tested including LH-RH, TSH, insulin, proinsulin, secretin, and glucagon

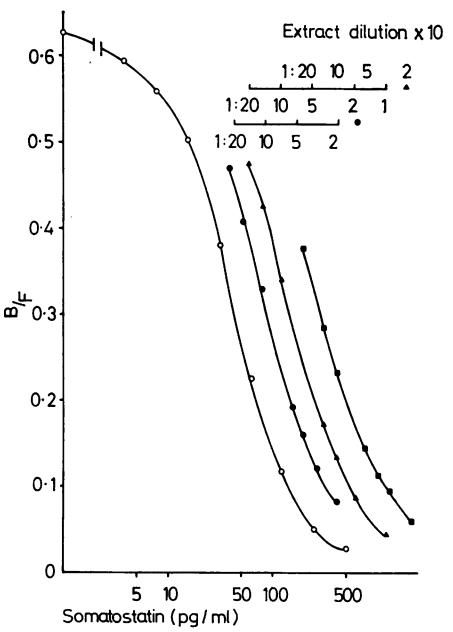

Fig. 2 *Displacement of ^{125}I-somatostatin by cyclic somatostatin and dilutions of tissue extracts. Cyclic somatostatin (○) or various dilutions of extracts from human pancreas (▲), stomach antrum (■) or corpus (●) were incubated with antibody VI/III/1 at a dilution of 1:12 500.*

(which share a common tetrapeptide with somatostatin), CCK, motilin, and gastrin at concentrations up to 1 μg. In a series of 10 assays intra-assay variations were 7% and 4%, and inter-assay variations 16% and 12%, for concentrations of 15 pg/ml and 125 pg/ml respectively. Dilutions of extracts from human stomach antrum or corpus, and pancreas (Fig. 2) and canine or rat extracts from stomach antrum and corpus, duodenum, and pancreas (not shown) were completely superimposable on the standard curves of all tested antibodies. Other cyclic somatostatin preparations and analogues show varying immunoreactive potency (Table 1).

TISSUE COLLECTION AND EXTRACTION

The mode of tissue collection was found to be important for all tissues except duodenum (Table 2). Significantly lower levels were routinely obtained when samples were allowed to remain at room temperature for relatively short periods of time. All subsequent studies were therefore performed with material which was immediately placed on dry ice. Careful control of the extraction procedure was necessary. Boiling the tissue in 0·2 M acetic acid for 15 minutes followed by homogenisation consistently gave the best results. Shorter periods of boiling or reversal of this procedure gave lower levels of somatostatin (Table 3). A number of further experiments were performed in order to validate the extraction procedure.

Extracts from rat liver or kidney contained undetectable amounts of somatostatin. Addition of synthetic somatostatin to liver and kidney (500 pg/mg) followed by extraction gave respective recoveries of 101 ± 3% and 75 ± 8% (n = 4). Rat corpus samples from fed rats extracted with either 0·2 M or 2 M acetic acid yielded levels of 109·3 ± 10·6 and 112·5 ± 13·4 ng/g tissue (n = 6) respectively. Hydrochloric acid/ethanol mixtures have been used for extraction of pancreatic islets, as this is the normal medium used for insulin extraction, and resulted in recoveries of 86·6 ± 1·1% (n = 10).

Experiments in which rat antrum was extracted and the sediment from the centrifugation twice re-extracted gave levels of 81·5 ± 15·2, 12·0 ± 3·0, and 1·9 ± 0·4 ng/g tissue (n = 4) for the three extractions. Assuming that the total extracted somatostatin approached 100%, then approximately 90% is removed by a single extraction. A human pancreas extracted by methods (c) and (d) of Table 3 and measured with three different antibodies gave values of 434, 428, and 420 ng/g tissue and 350, 280, and 330 ng/g tissue respectively.

Somatostatin concentrations in gastrointestinal tissues of starved rats are given in Table 4. Highest levels were found in stomach antrum and corpus, with lower levels in pancreas and duodenum. In the dog highest levels of somatostatin were found in the antrum and decreasing levels in the order: pancreas (head) > pancreas (processus uncinatus) = pancreas (tail) = stomach (corpus) > proximal duodenum (Table 2). The regional distribution in dog stomach measured in multiple biopsy specimens is shown in Fig. 3. Levels in the corpus were uniform throughout the greater curvature but were significantly lower in regions 3 and 7 of the lesser curvature. In the antrum, concentrations of somatostatin showed considerable variation between individual

Table 1 *Relative immunoreactive potencies of different somatostatin preparations*

	Relative immunoreactive potency (%)
Cyclic somatostatin (Serono)	100
Cyclic somatostatin (Kabi)	100
Cyclic somatostatin (Bioscience)	50
Tyr-1 somatostatin (Serono)	53
Tyr-11 somatostatin (Serono)	35
Linear somatostatin (Serono)	15
Des Ala^1-Gly^2-somatostatin (Serono)	14

Displacement curves with the polypeptides were plotted by Logit-Log transformation and the midrange dose (Logit $B/B_0 = 0$) obtained. Cyclic somatostatin (Serono) was taken as 100% (=28 f mol/tube) and relative potencies calculated for the other polypeptides.

Table 2 *Effect of collection procedure on somatostatin concentration in extracts of dog gastrointestinal tissues*

Collection procedure	*Somatostatin ng/g tissue (mean ± SEM)*					
	Stomach		*Pancreas*			*Duodenum*
	Antrum	*Corpus*	*Head*	*Processus uncinatus*	*Tail*	
A Dry ice	467 ± 139	189 ± 52	301 ± 68	195 ± 48	178 ± 14	97 ± 30
B Ice	493 ± 114	195 ± 33	333 ± 66	205 ± 41	187 ± 27	98 ± 17
C Room temp. 45 min	337 ± 71	148 ± 35	259 ± 59	138 ± 13	177 ± 35	91 ± 21
Number of animals	6	6	6	4	4	6

Tissue specimens were removed from dogs, immediately placed in vessels containing the substances shown (collection procedure), and subsequently stored at −20°C before extraction and assay as described in Methods section.

Table 3 *Extraction of somatostatin from human pancreas by different procedures*

Extraction procedure	*Somatostatin concentration (ng/mg protein)*		
	A		*B*
	1	*2*	
a. Boiled 2 min. Homogenised 2 min	1·35	2·21	1·96
b. Homogenised 2 min. Boiled 2 min	2·60	2·11	4·42
c. Boiled 15 min. Homogenised 2 min	24·48	19·23	39·00
d. Homogenised 2 min. Boiled 15 min	15·00	15·30	19·35

Specimens of human pancreas (90-150 mg) from two different subjects (A and B) were added to 0·2 M acetic acid (10 mg/ml) and subjected to the above extraction procedures. The mixture was then centrifuged, the supernatant lyophilised and assayed for somatostatin. Further details in Methods section. With pancreas A duplicate samples were extracted.

Table 4 *Somatostatin concentration in gastrointestinal tissues from starved rats*

Tissue	*Somatostatin concentration (ng/g tissue)*
Stomach-antrum	50·2 ± 4·9 (14)
Stomach-corpus	46·3 ± 3·1 (15)
Pancreas	33·4 ± 3·4 (14)
Duodenum	17·3 ± 1·3 (18)

Rats (200-300 g) were killed by cervical dislocation, the tissues removed and snap frozen on dry ice. Extraction was performed as described in Methods section. Values given are the mean ± SEM; numbers in parentheses indicate the number of rats.

dogs but the overall levels in all but region 11 were fairly constant. The level in sample 8 is intermediate between those of antrum and corpus, and was shown histologically to contain a mixture of both cell types.

Concentrations of somatostatin in human gastrointestinal tissues demonstrated a broad intersubject variation (Table 5). Human antrum from both control and duodenal ulcer patients contained similar mean levels to those found in the dog. At the present time the number of control corpus samples is too small to allow any definitive statement to be made as to whether there is any significant difference from duodenal ulcer patients. The mean level of somatostatin in histologically normal pancreas, despite the wide range, was similar to that in dog. Only a small group of patients with pancreatitis have been investigated and of these five had levels under 80 ng/g tissue and the remaining two had levels of 305 and 1425 ng/g tissue. In the extracts of pancreata from two children with neonatal hypoglycaemia due to nesidioblastosis, and the tissue from the one child having in addition a focal islet adenomatosis, extremely high concentrations of somatostatin were measured (Table 5).

GEL FILTRATION CHROMATOGRAPHY OF EXTRACTS

Gel filtration chromatography profiles from extracts of human antrum, corpus, and pancreas are shown in Fig. 4. A small peak, eluting in the region of the void volume, and a major peak eluting at the same position as tetradecapeptide somatostatin were present in all three chromatographic separations. This distribution is also seen with dog or rat gastrointestinal extracts. With two from 24 studied extracts heterogeneity has been observed in the region of tetradecapeptide somatostatin. Corpus

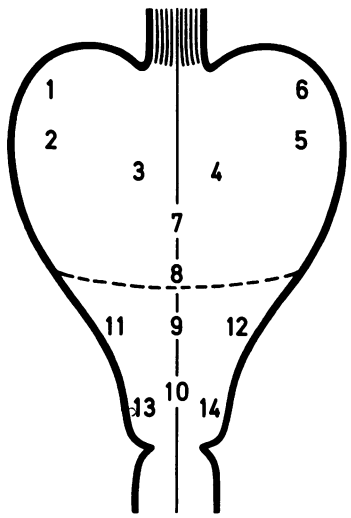

Fig. 3 *Somatostatin distribution in dog stomach. Biopsy specimens were removed from different regions of the dog stomach, snap frozen on dry ice and stored at −20°C. Extraction and assay as described in Methods section. Number of dogs = 4.*

Region	*Somatostatin concentration (ng/g tissue mean ± SEM)*	*Region*	*Somatostatin concentration (ng/g tissue mean ± SEM)*
1	124 ± 10	8	386 ± 215
2	131 ± 18	9	517 ± 130
3	60 ± 18	10	425 ± 50
4	113 ± 49	11	773 ± 254
5	98 ± 19	12	463 ± 213
6	141 ± 35	13	537 ± 181
7	65 ± 20	14	581 ± 181

Table 5 *Somatostatin concentration in human stomach and pancreas*

Organ	*Clinical state of patient*	*Somatostatin concentration ng/g tissue*	
		Mean ± SEM	*Range*
Stomach: antrum	Control (15)	465 ± 104	22-1500
	Duodenal ulcer (24)	614 ± 125	23-2250
Stomach: corpus	Control (6)	294 ± 144	65- 992
	Duodenal ulcer (16)	478 ± 170	19-2500
Pancreas	Control (15)	253 ± 43	31- 640
	Chronic pancreatitis (7)	270 ± 196	2-1425
	Neonatal hypoglycaemia (nesidioblastosis) (2)	(a) 3200; (b) 4840	
		Case a: adenomatosis 5500	

Biopsy specimens from stomach antrum or corpus and resected pancreas were collected and processed as described in Methods section. See also under this section for the clinical state of subjects classified as 'control'. The number in parentheses indicates the number of sample extracts.

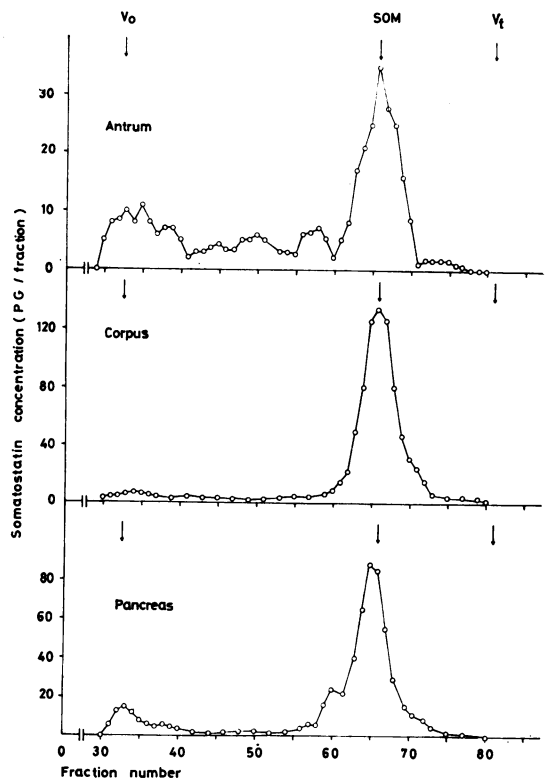

Fig. 4 *Gel-chromatography of extracts from human stomach antrum and corpus, and human pancreas. Stomach biopsy specimens from a patient with a duodenal ulcer and resected pancreas from a patient with chronic pancreatitis were extracted and processed as described in Methods section. Chromatography was performed on columns of Sephadex G25 fine (100 × 1 cm). Elution was with 0·2 M acetic acid at a flow rate of 5 ml/h. Collected fractions (1 ml) were lyophilised and dissolved in assay buffer. Void volume (Vo) and total volume (Vt) were determined using blue dextran and ^{125}I respectively. SOM indicates the elution position of synthetic cyclic somatostatin.*

from a patient with gastric ulcer and pancreas from a patient with chronic pancreatitis (Fig. 5) contained a further species eluting just before cyclic somatostatin. These peaks were reproducible on a second chromatography.

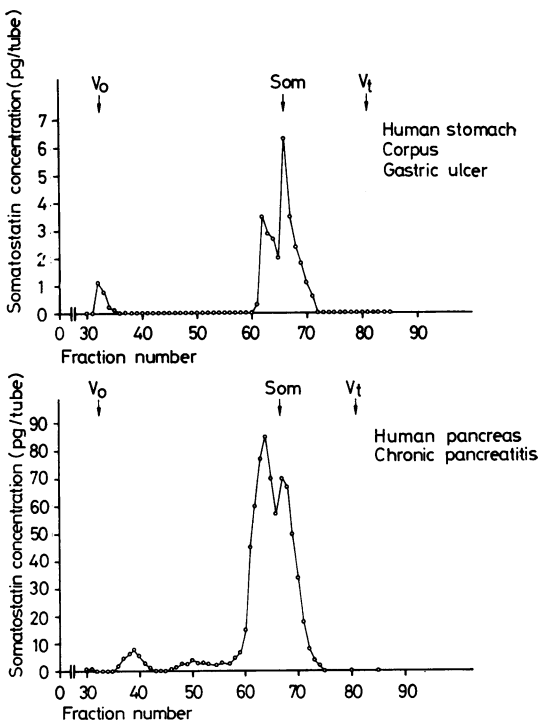

Fig. 5 *Gel-chromatography of human gastrointestinal extracts demonstrating heterogeneity in the region of tetradecapeptide somatostatin. Human stomach corpus extract from a patient with a gastric ulcer and pancreas extract from a patient with chronic pancreatitis were submitted to gel-chromatography as described for Fig. 4. Note the double peak in the region of tetradecapeptide somatostatin.*

Discussion

The presence of material in gastrointestinal tissues which is immunologically identical to somatostatin substantiates proposals that the observed suppression of hormone secretion and direct inhibition of different gastrointestinal target organs by this polypeptide are of physiological interest. The close proximity of somatostatin cells to G-cells in the antrum, and to A- and B-cells in the pancreatic islet, indicate a possible paracrine (Feyrter, 1953; Creutzfeldt, 1976) rather than an endocrine function. Evidence that pancreaticoduodenal vein plasma levels of somatostatin increase in response to glucose infusion, whereas peripheral levels remain constant, is in agreement with this suggestion (Schusdziarra *et al.*, 1977). However, further investigation of changes in tissue content and in circulating levels of somatostatin are needed and a highly sensitive radioimmunoassay is required. The increase in assay sensitivity compared to published methods (Arimura *et al.*, 1975a,b; Weir *et al.*, 1976; Kronheim *et al.*, 1976; Vale *et al.*, 1976) may be due to the characteristics of the antibody and the high specific activity of the labelled polypeptide. Vale *et al.* (1976) have analysed the binding characteristics of several antibodies raised against different antigens. Glutaraldehyde coupled cyclic somatostatin produced antibody sensitive to changes in amino acids Asn^5, Phe^7-Lys^9 and Phe^{11}, while antibody against bisdiazotized benzidine coupled Tyr^{11}-somatostatin detected changes in the N-terminal region Ala^1-Phe^6. Antigen produced by a carbodiimide coupling method similar to that described here gave antibody which reacted less strongly with analogues having changes in the region Asn^5-Trp^8 and Phe^{11}. Although only a limited number of analogues have been tested with the present antibody, both changes at Phe^{11} and at the N-terminus reduced the immunological potency, but the fact that linear somatostatin reacts to only 15% of the cyclic form suggests that conformational changes may also be important.

The method of collection and subsequent extraction of tissue is critical for validation of differences between tissues and in tissue content in response to stimuli. Rapid cooling of specimens on collection is evidently advisable, as even 45 minutes at room temperature is sufficient to give significantly reduced levels. In addition, a sufficient period of boiling before extraction is also important. This procedure is probably necessary in order to inactivate proteolytic enzymes but the cells may also be rendered more susceptible to disruption by homogenisation after boiling. The experiments designed to further validate that the majority of endogenous somatostatin was extracted by the present technique indicate that yields approached 90% after the single extraction and that 0·2 M or 2 M acetic acid, or acetic acid ethanol mixtures, give quantitatively similar yields. The somatostatin concentration in extracts from dog and rat showed the highest levels in stomach antrum and lowest levels in the duodenum. In the dog, stomach corpus, the processus uncinatus and tail of the pancreas contain equivalent amounts of somatostatin and the head of the pancreas is intermediate between antrum and these regions. Comparison of the levels found in rat tissues with published data reveals some discrepancies. Concentrations of 340 ng/g tissue (Vale *et al.*, 1976) and 142 ng/g tissue (Arimura *et al.*, 1975b) have been reported for rat pancreatic extracts, whereas in the present study the amount was 33 ng/g tissue. Similarly, extracts of stomach antrum and corpus, and duodenum gave lower values than those of Arimura *et al.* (1975b). Kronheim *et al.* (1976) found approximately 15-fold more somatostatin (ng/mg protein) in antrum as compared to the body of the stomach. As all published reports (including the present) appear to have used extracts from whole rat tissues rather than biopsy samples these disparities are likely to have other causes including the metabolic status of the animal before slaughter, differences in extraction technique, in antibody characteristics, and in the assay itself.

With the present assay system only a small amount of 'big somatostatin' (Arimura *et al.*, 1975b) is detected. Other workers have found varying amounts of 'big somatostatin' in extracts from normal pancreas (Weir *et al.*, 1976; Dupont and Alvarado-Urbina, 1976) and pancreatic tumour tissue (Larsson *et al.*, 1977). Evidence that it can be partially converted by urea treatment to a form eluting in a position identical to that of tetradecapeptide somatostatin indicates that it is probably either an aggregate of somatostatin or somatostatin non-covalently bound to a larger protein. The low amount detected by our assay suggests that the antigenic determinants are hidden in this form. However, at least part of 'big somatostatin' may be purely artefactual because of the relatively high protein content in the region of the void volume, particularly in unboiled extracts. Further heterogeneity of gastrointestinal somatostatin may, nevertheless, exist as shown for somatostatinoma extracts (Larsson *et al.*, 1977) and for the two examples presented here (Fig. 5). It remains to be resolved as to whether these species are produced only under pathological conditions and what relationship they have to immunoreactive somatostatin eluting in the position of the synthetic cyclic tetradecapeptide.

Direct comparison of the somatostatin concentration in dog and human tissues with those in rat is not possible, as the former were obtained with biopsy

specimens. The multiple biopsies taken from dog stomach show that there is a certain patchy distribution of D-cells and even neighbouring specimens may differ by 30-40% in content This may partially explain the large interindividual variation observed with human specimens. Nevertheless, the concentrations found in the different regions of the dog antrum (425 ± 50 to 773 ± 254 ng/g tissue) are of a similar level to those found in both duodenal ulcer patients and control subjects: 614 ± 125 and 465 ± 104 ng/g tissue respectively. Concentrations in human corpus from duodenal ulcer patients were higher than those in dog but, at present, it is not yet certain whether the difference from control subjects is significant owing to the small number of the latter group. Concentrations of somatostatin in pancreatic samples from patients with either a histologically normal pancreas or chronic pancreatitis also showed a wide distribution. This may be at least partly due to loss of some somatostatin owing to the time between operation and freezing of some of the samples and to the relative heterogeneity of the samples that were obtained (see: Methods—Collection procedures). The mean level (253 ± 43 ng/g tissue) in the 'normal' group is, however, similar to the concentration in an equivalent region of the dog pancreas (301 ± 68 ng/g tissue).

No definite statement can be made on the effect of chronic pancreatitis on somatostatin concentration because of the limited number of cases but, of the seven specimens studied, five had levels under 80 ng/g tissue, an amount found in only two of the control pancreata. The extremely high levels in the two children with nesidioblastosis (and surgically-proven islet cell hyperplasia, including B-, A- and D-cells) may be characteristic for the pancreas in neonatal hypoglycaemia but, as fresh pancreatic tissue of normal babies is not available for comparison, this cannot be verified.

It must be emphasised that, despite the immunological similarity between the material extracted from these tissues and hypothalamic somatostatin, confirmation of identity awaits purification and sequencing of the extrahypothalamic polypeptide. Nevertheless, as raised levels have been found in islets from streptozotocin diabetic rats (Patel and Weir, 1976) and release of immunoreactive somatostatin from isolated pancreatic islets and the perfused pancreas has been domonstrated (Schauder *et al.*, 1976, 1977a,b; Barden *et al.*, 1976; Patton *et al.*, 1976a,b), it is evident that somatostatin responds to functional changes in the pancreatic islet. Further investigations must concentrate on changes of the somatostatin tissue content in the gastrointestinal tract under normal and pathological conditions, and substantiate the reported stimulation of local blood levels in response to physiological stimuli.

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