# Circulating Prosomatostatin-derived Peptides

## Differential Responses to Food Ingestion

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## Abstract

**Prosomatostatin (pro-S) and its bioactive posttranslational products, somatostatin-14 (S-14), somatostatin-13 (S-13), and somatostatin-28 (S-28), were measured in human plasma by the use of immunoglobulins to the $NH_2$-terminus of S-28 conjugated with agarose to separate them and, thereafter, by RIA with an antiserum recognizing the COOH-terminus of pro-S, and by specific RIA for the $NH_2$-terminus of S-14 and pro-S. In healthy men, mean basal levels of pro-S were 4 pg equivalent S-14/ml; S-14/S-13 combined were 9 pg equivalent S-14/ml; and S-28 levels were 16 pg/ml. After a 700-kcal meal, pro-S, S-14, and S-14/S-13 did not change, whereas S-28 levels doubled by 120 min and remained elevated for 240 min. To evaluate the origins of these peptides, their levels were compared in peripheral, portal, gastric, and mesenteric veins of anesthetized patients and in patients with total resection of stomach and pancreas before and after nutrient intake. The stomach and small intestine were sources of both peptides; however, most S-28 originated in the small intestine. These findings suggest that, in contrast to S-14, S-28 is a hormone and may modulate postprandial nutrient absorption and use.**

## Introduction

The tetradecapeptide somatostatin (S-14)[1] has stimulated much interest because it inhibits diverse metabolic functions (1). Relevant to fuel homeostasis, S-14, in pharmacologic doses, decreases the secretion of several peptides from the gastrointestinal (GI) tract and pancreas and alters intestinal peristalsis (1, 2). Because S-14 is secreted from cells that are widely distributed, notably in the central and peripheral nervous systems, pancreas, and GI tract (3–6), the prevailing belief is that it acts on adjacent cells to modulate the release of specific secretory products (7, 8). On the other hand, somatostatin-like immunoreactivity (SLI) is present in mammalian plasma (9–13), and rises after ingestion of nutrients or infusion of supraphysiologic doses of neurotransmitters and selected GI peptides (14–19). From immune neutralization studies in dogs and S-14 infusions into man mimicking postprandial levels of SLI, Schusdziarra et al. (20) and Zyznar and colleagues (21), postulated that circulating S-14 acts as a hormone that may be involved in nutrient assimilation.

SLI in human plasma is composed of a heterogeneous group of substances, including a protein of large molecular weight, termed "big plasma somatostatin," which is thought to be an immunoglobulin (22), and three peptides cleaved from their precursor, prosomatostatin (pro-S). These include somatostatin-28 (S-28), a 28-amino acid–containing peptide incorporating S-14 at its COOH-terminus, S-14, and des-Ala S-14(S-13) (23). Because pro-S is processed to either S-28 or S-14 in different cells (24–26), it is plausible that they fulfill divergent roles. Their precise measurement has been hindered by the use of nonspecific RIA directed to the central region of S-14, precluding their independent quantification in native plasma. S-28 can be separated from S-14 and S-13 by gel filtration and substances with similar elution profiles have been found in plasma of the rat, dog, man, and pig (27–31). Increased plasma levels of peptides coeluting with S-28 and S-14 in response to food intake in man have been reported (32, 33). However, further understanding of the secretion and biologic roles of pro-S related peptides has been hampered by difficulties in carrying out repetitive gel filtration of small amounts of plasma and the use of nonspecific RIA. Therefore, we developed polyclonal antisera against different regions of pro-S and used the antibodies for selective immunoadsorption and RIA. In conjunction with an RIA specific for S-14, separation by gel filtration, and HPLC, these methods have enabled us to identify and measure pro-S and its COOH-terminal cleavage products and determine if they are differentially released after nutrient ingestion in men. To assess indirectly the major sources of circulating pro-S-derived peptides from the GI tract, we have quantified their basal and postprandial levels in patients with total gastrectomy and total pancreatectomy and in venous plasma from regions of the stomach and small intestine in man.

## Methods

*Peptides.* $Tyr^{14}$-S-28[1-14], $Tyr^{1}$-S-14, and $Tyr^{11}$-S-14 were purchased from Peninsula Laboratories, San Carlos, CA. S-14, S-13, S-12, and analogues of S-14 in which alanine or tyrosine was substituted for residues at the 5, 6, 8, and 10 positions were gifts from Dr. J. Rivier, Salk Institute, La Jolla, CA. S-28 was donated by Dr. L. Pradayrol, Karolinska Institute, Stockholm, Sweden, and purchased from Peninsula Laboratories. S-28[1-12], S-28[1-14], and 13 analogues, in which the amino acids were sequentially deleted from the $NH_2$-terminus of S-28, were kindly provided by Dr. N. Ling and Dr. R. Guillemin, Salk Institute. Pro-S was partially purified from media from a propagated rat medullary cell carcinoma cell line (34). The 13-amino acid $NH_2$-terminal peptide of pro-S (pro-S-NTP) and $Try^{14}$-pro-S-NTP were

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1. *Abbreviations used in this paper:* AS, antisera; GI, gastrointestinal; HAc, acetic acid; pro-S, prosomatostatin; pro-S-NTP, $NH_2$-terminal peptide of pro-S; S-13, somatostatin-13; S-14, somatostatin-14; S-28, somatostatin-28; TFA, trifluoracetic acid; Tg, thryoglobulin.

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custom synthesized at Peninsula Laboratories. The tyrosinated peptides (1–5 μg) were coupled with $^{125}$I Na (Amersham Corp., Arlington Heights, IL) at pH 10 by a lactoperoxidase method (35). The radiolabeled peptides were separately filtered through a 1 × 40 cm column of Sephadex G-25 (Fine) (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated in 0.1 N acetic acid (HAc) containing 0.1% BSA (Miles-Pentex, Kanakakee, IL) at 15–20 ml/h. The column was calibrated with blue dextran with molecular mass of > $1 \times 10^6$ D and $^3H_2O$ (New England Nuclear, Boston, MA). The elution positions were described by the coefficient of distribution $K_d = (V_e - V_o)/V_i$. The radioactive peptides reacting optimally with the specific antisera were eluted with the following $K_d$: $^{125}$I-Tyr$^{14}$-S-28(1-14), $K_d = 0.73$; $^{125}$I-Tyr$^1$-S-14 and $^{125}$I-Tyr$^{11}$-S-14, $K_d = 1.10$. The tracers were then diluted in appropriate buffers and stored at −20°C.

*Antisera.* Tyr$^{11}$-S-14 and S-14 were separately linked to thyroglobulin (Tg) by dissolving 5 mg in 5 ml of sterile NaCl containing 20 mg bovine Tg and 920 mg carbodiimide (25). The solutions were dialyzed and frozen at −20°C. Diazo compound was synthesized as follows: 18 mg of benzidine were dissolved in 0.4 ml warm HCl solution (2.4 ml 11.6 N HCl in 15 ml $H_2O$) and 0.1 ml of Na nitrite solution (69 mg in 0.5 ml $H_2O$) was added. After 5 min at 4°C, a saturated solution of sulfamic acid (60 μl) was introduced resulting in 6 mg of diazo compound per 100 μl. 5 mg of Tyr$^{11}$-S-14 were coupled with tyrosines in 10 mg Tg dissolved in 1 ml of 130 mM NaCl in 160 mM Na borate buffer, pH 9, by addition of 15 μl of diazo compound (0.8 mg) at 4°C for 2 h. The conjugates were extensively dialyzed in water and lyophilized. Based on distribution of free and bound $^{125}$I-Tyr analogues, we calculated that 25–30% of the peptides were linked to Tg. Approximately 30 μg of peptide was emulsified in CFA and injected intradermally at 10–20 sites into each of 10 young female New Zealand white rabbits. Boosters of the same preparations were given every 3 mo. The antisera were tested for titer, specificity, and avidity by measuring the binding of the different iodinated peptides by RIA. The following trivial designations were assigned to the antisera (AS) selected for RIA: F-4, which was obtained with Tyr$^{14}$-S-28(1-14) joined with Tg by carbodiimide; AS-77, which was made with Tyr$^{11}$-S-14 coupled to Tg by diazotization; AS-10, which was generated by linking S-14 to Tg with carbodiimide; and Kiisha, which was obtained by conjugation of Tyr$^{14}$-pro-S-NTP to limpet hemocyanin with carbodiimide (34).

*RIA.* The optimal conditions for equilibrium binding of the radioligands with their respective antisera were (*a*) F-4, AS-77, and Kiisha: 50 mM barbital buffer, pH 8.0; (*b*) AS-10: 130 mM borate buffer, pH 8.5. Each buffer contained 0.25% BSA and thiomerosal (1:10,000 final concentration). Assays were carried out by mixing 0.9 ml buffer with 0.05 ml of diluted antiserum to achieve final concentrations of 1:8,000 for F-4, 1:60,000 for Kiisha, 1:4,000 for AS-77, and 1:100,000 for AS-10. Variable volumes of solution containing either reference standards or unknown samples were added. Inhibitors of proteolytic enzymes were not routinely included, as degradation of peptides was not found with processed plasma samples. After incubation for 24 h at 4°C, 0.05 ml of $^{125}$I-tyrosinated peptides was added and the reaction carried out for an additional 48 h. In the AS-10 RIA, free and antibody-bound labeled peptides were separated by addition of 1 ml of a suspension of 1% activated charcoal (Norit A; Eastman-Kodak, Rochester, NY) in 130 mM borate buffer, pH 8.5; whereas, with all the other peptides, the bound tracer was precipitated with 1 ml of 30% polyethylene glycol (Carbowax 8000; Great Western Chemical, Seattle, WA) after addition of 1 mg of partially purified bovine gamma globulin (fraction 2; Sigma Chemical Co., St. Louis, MO).

*Blood collection and processing of plasma.* To circumvent spurious measurements of peptides in native plasma as a result of degradation of endogenous peptides and tracers as well as nonspecific factors such as "big plasma somatostatin", we examined the effects of extremes of pH on the activities of both endo- and exopeptidases in plasma and thereafter, elimination of macromolecules. $^{125}$I Tyr$^{11}$-S-14 and $^{125}$I-Tyr$^1$ S-14 were used to monitor serine protease and aminopeptidase mediated degradation, respectively, by measurement of radioactivity in supernatants after addition of 10% TCA. Below pH 5.3, neither tracer was destroyed between 4 and 37°C throughout 30 min. Based on a series of experiments to rigorously test this question, we have adopted the following procedure. Blood was collected in tubes containing heparin (1,000 U/10 ml blood), placed in ice, and centrifuged to remove the red cells within 10 min. The plasma was adjusted to pH 3 by addition of 1 N HCl. Under these circumstances, no degradation of either tracer in plasma was found at 22°C for up to 8 h. Recovery of authentic S-14 and S-28 separately added to plasma, kept at room temperature for 6 h, and subsequently stored for 2 mo at −20°C exceeded 90% ($n = 5$).

For routine analyses, 5-ml aliquots of acidified plasma were filtered through cartridges containing octadecylsilyl silica (Sep-Pak C-18, Waters Associates, Milford, MA) after serial washing with absolute methanol and deionized water. Plasma proteins including enzymes and big plasma somatostatin were removed by washing sequentially with 5 ml of water and 5 ml of 0.1% trifluoroacetic acid (TFA) in water. The retained peptides were eluted with 5 ml of a solution of 80% methanol and 1% TFA. The eluate was air dried and dissolved in 2 ml of assay buffer. Recoveries of authentic S-14, S-13, and S-28, (50 and 100 pg) added to 5 ml of buffer or of 5 ml of acidified plasma previously stripped of endogenous peptide by passage through SepPak, were 78±2% ($n = 15$) and did not differ significantly from each other.

*Affinity chromatography.* Immunoglobulins from the F-4 and AS-10 antisera were partially purified by precipitation with 33% $(NH_4)_2SO_4$. Each precipitate was dissolved in 100 mM borate buffer containing 150 mM NaCl, pH 7.0, dialyzed extensively against this buffer, and lyophilized. 200 mg protein was coupled to 5 g of cyanogen bromide–activated Sepharose 4B. Residual active groups on the agarose were blocked by washing with 20 ml of 200 mM glycine buffer, pH 8.0. After repetitive sequential washing with 100 mM $NaHCO_3$-500 mM NaCl solution, pH 8.3, and 100 mM Na acetate-500 mM NaCl buffer, pH 4.0, the gel was suspended in 130 mM borate buffer, pH 8.5, and 3 ml (equivalent to 1 g dry gel) were poured into 5 ml syringes (1 × 5 cm). Reference peptides or unknowns, diluted in 2 ml borate buffer, were introduced into the syringe. The gel was washed with 40 ml of borate buffer and the first 10 ml was saved (fraction 1) and stored at −20°C. The gel was then washed with 10 ml of 0.2 N HAc, pH 3.5, containing 0.2% BSA. The first 5 ml was collected, lyophilized, and reconstituted in 130 mM borate buffer containing 0.25% BSA (fraction 2).

*Gel permeation chromatography.* Chromatography was carried out in columns (1.5 × 90 cm) containing BioGel P-10 (200-400 mesh) (BioRad Chemical Division, Richmond, CA) equilibrated in 50 mM borate buffer, pH 8.5 containing 0.25% BSA. The columns were calibrated with blue dextran with molecular mass exceeding $1 \times 10^6$ D; cytochrome *c* (Sigma Chemical Co.), molecular mass 14.5 kD; pork insulin (Eli Lilly & Co., Indianapolis, IN), molecular mass 6.1 kD; S-28, molecular mass 3.1 kD; and S-14, molecular mass 1.6 kD, and $^3H_2O$. Filtration occurred under gravity at room temperature at 10 ml/h and 1-ml fractions were automatically collected.

*HPLC.* Chromatography was performed on a modular system consisting of a solvent delivery system with a dynamic mixer (model 8700; Spectra-Physics, San Jose, CA), an injector (model U6K; Waters Associates) and an absorbance detector (model 757, Spectraflow; Spectra-Physics). 0.25–1.0-ml samples were injected on to a micron analytical column (Vydac C-18 5; The Separations Group, Hesperia, CA). The gradients consisted of 5–25% acetonitrile ($CH_3CN$)/0.1% TFA stepped over 20 min, followed by 25–37% $CH_3CN$/0.1% TFA linear gradient for 70 min, a 37–50% $CH_3CN$/0.1% TFA linear gradient for 10 min and a 50–90% $CH_3CN$ linear gradient for 10 min. The flow rate was 0.5 ml/min. Fractions were collected at 1-min intervals for 60 min, dried on a concentrator, (model SVC-200; Savant Speed Vac, Farmingdale, NY), and reconstituted in assay buffer.

*Subjects.* The volunteers included 30 healthy men (age range 22–26 yr) who were within 10% of ideal body weight; two men aged 49 and 65 yr with resection of the entire stomach as treatment for Zollinger-Ellison syndrome; a 55-yr-old woman with total pancreatectomy for ductal carcinoma; and a 36-yr-old woman with complete resection of the stomach, pancreas, and duodenum because of multiple endocrine

adenomata. None of the healthy men admitted to taking medication. The patients with total gastrectomy and total pancreatectomy did not have active disease. The pancreatectomized patients were receiving insulin as treatment of their diabetes and pancreatic enzymes were taken with each meal.

The subjects gave informed consent before participation. After an overnight fast, they were admitted to beds on the Clinical Research Center. An indwelling line was inserted into an antecubital vein and blood was withdrawn at 30-min intervals before and up to 4 h after nutrient intake. All subjects ingested a liquid meal consisting of 95 g carbohydrate, 26 g protein, and 25 g fat, totaling 700 kcal in 700 ml (Ensure Plus; Ross Laboratories, Columbus, OH). The normal men swallowed the meal within 3–5 min; whereas, the patients ingested it by 20–30 min. The pancreatectomized patients received their typical dose of insulin and took their usual dose of pancreatic enzymes before eating. Five healthy men also ate a standard breakfast of solid food of comparable calories and nutrient distribution.

5 ml blood was obtained from the two gastric, superior mesenteric, portal, and antecubital veins in four obese women undergoing gastric plication as treatment for their obesity. The sampling was random

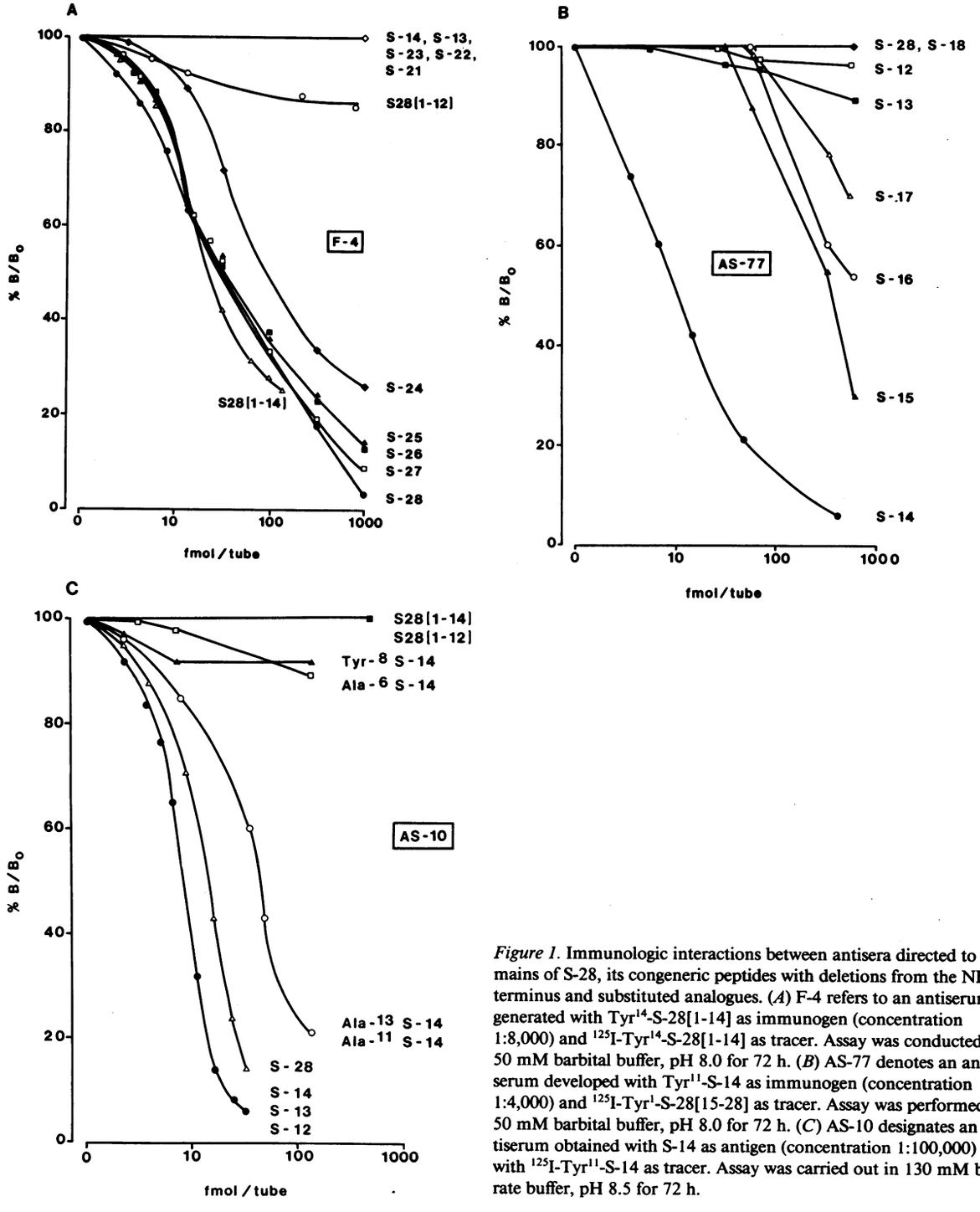


*Figure 1.* Immunologic interactions between antisera directed to domains of S-28, its congeneric peptides with deletions from the $NH_2$-terminus and substituted analogues. (*A*) F-4 refers to an antiserum generated with $Tyr^{14}$-S-28[1-14] as immunogen (concentration 1:8,000) and $^{125}I$-$Tyr^{14}$-S-28[1-14] as tracer. Assay was conducted in 50 mM barbital buffer, pH 8.0 for 72 h. (*B*) AS-77 denotes an antiserum developed with $Tyr^{11}$-S-14 as immunogen (concentration 1:4,000) and $^{125}I$-$Tyr^{1}$-S-28[15-28] as tracer. Assay was performed in 50 mM barbital buffer, pH 8.0 for 72 h. (*C*) AS-10 designates an antiserum obtained with S-14 as antigen (concentration 1:100,000) with $^{125}I$-$Tyr^{11}$-S-14 as tracer. Assay was carried out in 130 mM borate buffer, pH 8.5 for 72 h.

from the different sites and all samples were obtained within 10 min of one another.

*Statistical analysis.* Data were analyzed by the *t* test, using a statistical program on CLINFO, and $P$ values $< 0.05$ were considered significant.

## Results

*Characterization of antisera against regions of pro-S.* The binding characteristics of F-4 antiserum with S-28 and peptides with amino acid deletions from the $NH_2$-terminus of S-28 are shown in Fig. 1 *A*. This antiserum interacted not only with S-28(1-14) but also recognized S-28, S-27, S-26, and S-25 with equal avidity. S-24 reacted less well and all other peptides did not interact with this antiserum. These findings were confirmed by their behavior when filtered through a column of agarose-F-4 conjugate. S-28 and analogues through S-24 were retained, whereas S-23 and peptides with further $NH_2$-terminal deletions and pro-S traversed the immunoadsorbent unhindered. Therefore, we infer that the epitope with which this antiserum reacted involved the $Asn^5$-$Pro^6$ sequence of S-28. Of note, the cross-reactivity of this antiserum with S-28(1-12) was extremely poor, and pro-S did not bind, implying that modifications of their secondary structure also affected binding. The lowest limit of detection of S-28 with F-4 was 2 fmol per tube, with interassay and intraassay variances of 15 ($n$ = 10) and 8% ($n$ = 10), respectively.

In Fig. 1 *B* are displayed the specificities of antiserum AS-77 obtained by orienting the S-14 molecule towards its $NH_2$-terminus by Tyr-diazo-Tyr linkage at position 11. From displacement curves with the various peptides, $Ala^1$ and $Gly^2$ appear to be the critical determinants. Based on the sensitivity of the displacement curves and the lack of binding of pro-S, S-28, and other analogues to bind with this antiserum at other than inordinately high concentrations, for practical purposes in measurement, AS-77 was considered specific for S-14. The minimum of S-14 detected with AS-77 was 2 fmol per tube, with interassay and intraassay variances of 10 ($n$ = 10) and 8% ($n$ = 10), respectively. The interactions of S-28, related peptides, and analogues with antiserum AS-10 are shown in Fig. 1 *C*. This immune serum was judged to interact with the $Phe^7$-$Trp^8$-$Lys^9$ residues of S-14. Because it bound peptides in the COOH-terminal region of pro-S including S-28, AS-10 is not specific for S-14. From stoichiometric comparisons, S-13, S-12, and S-14 interacted equivalently, whereas, S-28 had only one-third of the avidity of S-14, from which we infer that the structural configuration imparted by the $NH_2$-terminal amino acids in SS-28 partially occluded ligand-antibody interactions. The minimum reliable threshholds for detection of S-14 and S-28 with AS-10 were 1 fmol (2 pg) per tube and 1 fmol (3 pg) per tube, respectively, with interassay and intraassay variances of 8 ($n$ = 10) and 6% ($n$ = 10) for each. The characteristics of the assay for pro-S-NTP and its reactivity with pro-S have been previously reported (34).

*Characterization of pro-S-related peptides in plasma.* After acidified plasma samples were processed through Sep-Pak, the peptides were measured either directly by AS-10 and AS-77 RIA or after their separation by region specific immunoadsorbents and gel filtration (see Methods). Because F-4 antiserum interacted only with peptides from S-28 to S-24, it was coupled to agarose to separate S-28 from S-14 and S-13. Recoveries of authentic S-14 and S-13 added to buffer or acidified plasma, processed through SepPak and passed freely through the immunoadsorbent (fraction 1) were 80±3% ($n$ = 10), whereas, S-28 through S-24, which were retained by the immunoadsorbent and eluted with 0.2 N HAc plus 0.2% BSA (fraction 2), were recovered with an efficiency of 50±5% ($n$ = 10). Therefore, values in both fractions 1 and 2 were corrected to 100%.

To determine if the endogenous peptides separated by this technique corresponded with pro-S and its processed peptides,

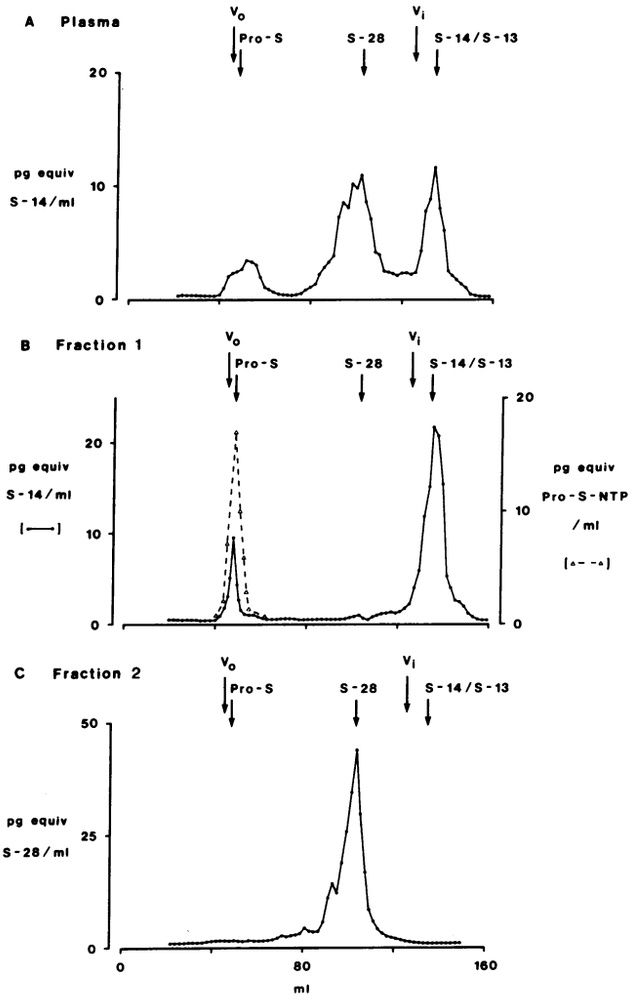


*Figure 2.* Profiles of pro-S and related peptides separated by gel permeation chromatography. Filtration was carried out in a 1.5 × 90 cm column containing Biogel P-10 (200–400 mesh) in 50 mM borate buffer, pH 8.0 containing 0.25% BSA. Measurements were by RIA with AS-10 antiserum with either S-14 or S-28 as standards and by Kiisha antiserum with pro-S-NTP as standard. Arrows designate peaks where markers of known molecular weight were eluted. (*A*) Peptides from 50 ml plasma from a healthy man were adsorbed and subsequently eluted from SepPak. (*B*) Fraction 1 designates the fall-through of peptides from the immunoadsorbent containing immunoglobulins from F-4. Plasma (50 ml) was adsorbed to SepPak, eluted, and applied to the immunoadsorbent. (*C*) Fraction 2 designates the peptide(s) adherent to the immunoadsorbent containing immunoglobulins from F-4 antiserum. The peptides from 50 ml of plasma were adsorbed to SepPak, eluted, and applied to the immunoadsorbent. The retentate was eluted with the 0.2 N HAc plus 0.2% BSA, lyophilized, and reconstituted in buffer. Points represent the mean of six separate experiments.

50–500-ml aliquots of human plasma were adjusted to pH 3, the peptides were adsorbed onto SepPak, and the eluates submitted to gel filtration or HPLC, either directly or after their separation as fractions 1 and 2 from the immunoadsorbent. When the peptides adsorbed to SepPak from acidified plasma were eluted and directly filtered on Biogel P-10, three major peaks were detected by AS-10 RIA (Fig. 2 *A*). The identity of the front-running peak eluted at $V_o$ has not been established; however, partially purified pro-S from the media of a cultured rat medullary thyroid carcinoma cells coeluted with this peak. The peptide in this eluate also registered in the Pro-S-NTP RIA, and all the immunoreactivity was retained on the AS-10 immunoadsorbent indicating that this peak contained pro-S. Lacking purified pro-S for comparison of avidity for AS-10 antiserum, we could not precisely compare the magnitude of the $V_o$ peak with the other two peaks, which were quantified with S-28 and S-14 as standards. With S-14 as standard, it composed one-eighth of the total peptides, and approximated 20% of the peak coeluting with S-14/S-13. The immunoreactivity in the remaining peaks coincided with the $K_d$ of authentic S-28 and S-14/S-13, respectively. In Fig. 2, *B* and *C* are shown the behavior of the peptides separated into fractions 1 and 2 from the F-4 immunoadsorbent measured by AS-10 RIA. Two peaks in fraction 1 corresponding to those found in native plasma had $K_d$'s similar to partially purified rat pro-S and S-14/S-13. The peak attributable to S-28 in nonfractionated plasma was no longer demonstrable. Of note, S-13 could not be distinguished from S-14 by gel filtration. In fraction 2, a peak with the $K_d$ for authentic S-28 was consistently found. To assess further the homogeneity of the immunoreactivity retained in fraction 2 from the F-4 immunoadsorbent, we collected 500 ml of blood into syringes containing 500 mM $Na_2HPO_4$ (15:25 vol/vol) to keep pH at 5.2 (the minimum pH without hemolysis), separated red cells by centrifugation and further lowered the pH to 3 to minimize action of aminopeptidases and serine proteases. After processing by SepPak and

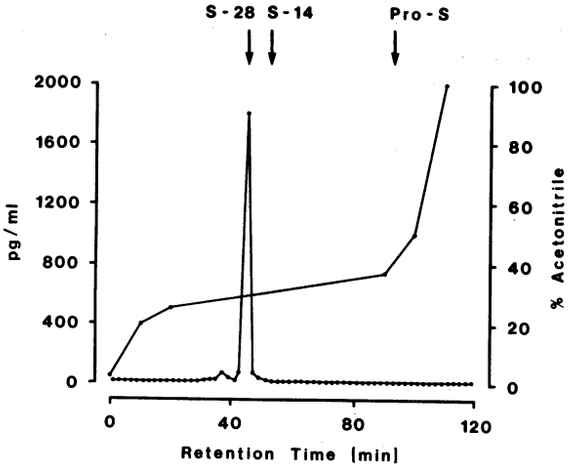


*Figure 3.* HPLC profile of fraction 2 from the F-4 immunoadsorbent. Peptides from 500 ml of plasma were processed by SepPak and F-4 immunoadsorbent. The retentate, eluted in 0.2 N HAc plus 0.2% BSA, was lyophilized, concentrated by SepPak, and applied to C-18 reverse-phase column with a 5–90% $CH_3CN$/0.1% TFA linear gradient. The eluates were analyzed by AS-10 RIA. *Arrows* refer to retention times of authentic standards.

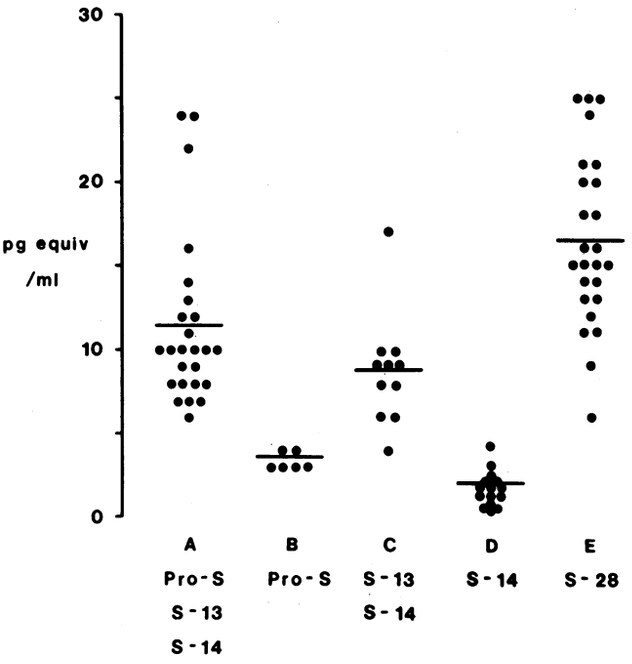


*Figure 4.* Levels of pro-S-derived peptides in plasma from healthy men after an overnight fast. (*A*) Peptides from plasma (5 ml) were adsorbed and eluted from SepPak and measured in fraction 1 from the F-4 immunoadsorbent by AS-10 RIA. ($n$ = 25). (*B*) Peptides from plasma (50 ml) were adsorbed and eluted from SepPak and subjected to Biogel P-10 chromatography. Peak 1 was segregated, concentrated, and analyzed by AS-10 RIA ($n$ = 6). (*C*) Peptides from plasma (50 ml) were adsorbed and eluted from SepPak and subjected to Biogel P-10 chromatography. Peak 3 (Fig. 2 *C*) was segregated, concentrated, and analyzed by AS-10 RAI ($n$ = 11). (*D*) Blood (50 ml) was collected directly into 500 mM $Na_2HPO_4$, final pH 5.2, the red cells separated, the pH adjusted to 3 and peptides from plasma were adsorbed, eluted from SepPak, and analyzed by AS-77 RIA ($n$ = 15). (*E*) Peptides from plasma (5 ml) were adsorbed and eluted from SepPak and measured in fraction 2 from the F-4 immunoadsorbent by AS-10 RIA ($n$ = 34).

separation of fraction 2 by F-4 immunoadsorption the fraction underwent HPLC. As shown in Fig. 3, a minor (< 5%) and major peak measured by AS-10 RIA were found. The latter had a retention time coincident with that of authentic S-28.

*Basal levels of pro-S-derived peptides in man.* Levels of pro-S and related peptides, in combination and after separation from each other, were obtained in plasma of healthy young men after an overnight fast. The mean basal level of the combined peptides in plasma after separation of big plasma somatostatin by means of SepPak and analysis by AS-10 RIA was 15±0.8 (SE) pg equivalent S-14/ml ($n$ = 35). In Fig. 4 are shown the mean and range of values for the peptides using different methods for their separation. In Fig. 4 *A* are recorded the combined levels of peptides in fraction 1 from the F-4 immunoadsorbent. Mean values of 12 pg equivalent S-14/ml include pro-S, S-14, and S-13. To measure their separate contributions, plasma was passed through SepPak, the retained peptides eluted, filtered through Biogel P-10 and peaks 1 and 3 corresponding to pro-S and S-14/S-13, respectively, were segregated, concentrated by SepPak and assayed by AS-10 RIA. (Recoveries of standards were 72±5% and unknowns were appropriately corrected.) Mean levels for pro-S were 4 pg equiva-

lent S-14/ml (Fig. 4 *B*) and S-14 and S-13 together were 9 pg equivalent S-14/ml (Fig. 4 *C*). To separately measure S-14 we used AS-77 RIA, which discriminates between S-14 and S-13. Aminopeptidase mediated cleavage of $NH_2$-terminal alanine of S-14 during plasma collection and processing was minimized by aspirating blood into chilled syringes containing 500 mM $Na_2HPO_4$ to achieve a final pH of 5.2, removing the red cells, and adjusting plasma to pH of 3. The peptides were then separated from plasma enzymes by SepPak and assayed (Fig. 4 *D*). The mean value of 2 pg S-14/ml was significantly lower ($P < 0.005$) than the mean level of 9 pg equivalent S-14/ml for S-14/S-13 combined in peak 3 by gel filtration. Thus, we interpret these differences as reflecting the contribution of S-13 in this peak. In Fig. 4 *E* is displayed the range of S-28 values separated in fraction 2 by the F-4 immunoadsorbent. The mean level was 16 pg/ml measured by AS-10 RIA using S-28 as standard. Of note, the mean basal levels of 12 pg equivalent S-14/ml for fraction 1 together with the 16 pg equivalent S-28/ml in fraction 2 exceed the mean of 15 pg equivalent S-14/ml in nonfractionated plasma. This apparent discrepancy is due to the lack of stoichiometry in interaction of these peptides with AS-10 antiserum. Because S-28 is only one-third as avid as S-14 in displacement, the values for S-28 require division by three to correct for its contribution in nonfractionated plasma.

*Plasma levels of pro-S-derived peptides after food intake.* In Fig. 5 are compared the levels of pro-S and S-14/S-13 in plasma from men before and after the ingestion of a liquid meal of mixed composition. Mean basal levels of pro-S, S-14 and S-13 in fraction 1 from the F-4 immunoadsorbent in 18 subjects were 14 pg equivalent S-14/ml and did not change throughout 4 h after food intake (Fig. 5 *A*). In five individuals studied separately, S-14 levels, quantified by AS-77 RIA after processing blood collected in 500 mM $Na_2HPO_4$, pH 5.2, were also unaltered after food intake (Fig. 5 *B*). Levels of pro-S measured as pro-S NTP equiv, segregated in fraction 1 from the F-4 immunoadsorbent and thereafter retained by the AS-10 immunoadsorbent were not significantly perturbed from baseline after the liquid meal. In contrast, as shown in Fig. 6, concentrations of S-28 separated in fraction 2 from the F-4 immunoadsorbent in 18 volunteers rose from a baseline of 24 pg/ml by 30 min after food intake, reached a zenith of 44 pg/ml between 120 and 180 min ($P < 0.001$) and declined by 4 h. These findings were confirmed by measurement of the peptides coeluting with authentic S-28 on Biogel P-10 (peak 2, Fig. 3) in whom a doubling from fasting levels occurred by 120 min. Similar changes in S-28 but not in pro-S, or S-14/S-13 levels were obtained in five subjects who ate a solid meal of comparable calories.

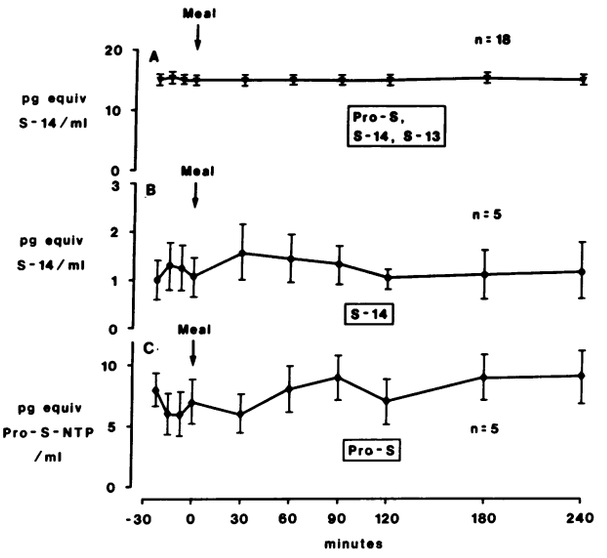


*Figure 5.* Levels of pro-S and related peptides in plasma from healthy men before and after intake of a liquid meal (Ensure Plus, 700 kcal). (*A*) Peptides from plasma (5 ml), adsorbed and eluted from SepPak, were measured in fraction 1 from the F-4 immunoadsorbent by AS-10 RIA. (*B*) Blood (50 ml) were collected directly into 500 mM $Na_2HPO_4$, final pH 5.2, the red cells were separated, the pH was adjusted to 3, and peptides were adsorbed and eluted from SepPak and analyzed by AS-77 RIA. (*C*) Peptides from plasma (10 ml) were adsorbed and eluted from SepPak and applied to the F-4 immunoadsorbent. Fraction 1 was segregated, concentrated and applied to an AS-10 immunoadsorbent. The retained fraction eluted in acid was lyophilized, dissolved in buffer, and assayed by pro-S NTP RIA.

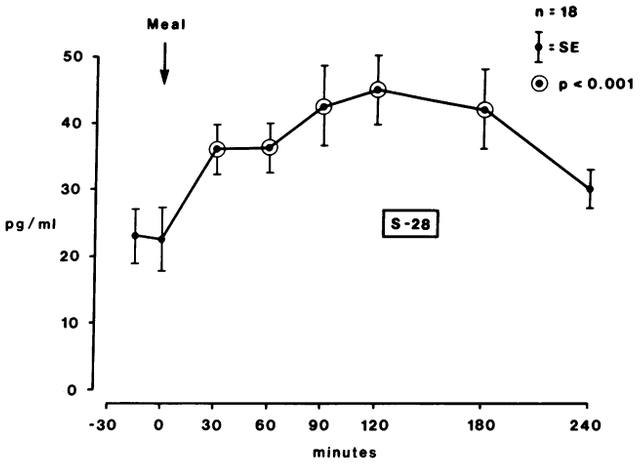


*Figure 6.* Levels of S-28 in plasma before and after intake of a liquid meal (Ensure Plus, 700 kcal). Peptides from plasma (5 ml) were adsorbed and eluted from SepPak and measured in fraction 2 from the F-4 immunoadsorbent by AS-10 RIA.

*Levels of pro-S-related peptides in venous effluent from the GI tract in man and responses to nutrients in patients with surgical resection of stomach and pancreas.* The levels of peptides measured by AS-10 RIA in fractions 1 and 2 from the F-4 immunoadsorbent in plasma from selected venous drainage sites of the GI tract in four obese women during surgery are shown in Table I. Concentrations of peptides in fraction 1 ranged from 9 to 35 pg equivalent S-14/ml in peripheral veins, and, in each individual, they were greater in the portal vein. No differences were seen between levels in the superior mesenteric vein and the portal vein. In contrast, levels of in both the right and left gastric veins were greater than in the portal vein in each subject. Concentrations of S-28 in fraction 2 were higher in all sites than those for pro-S and S-14/S-13, in combination, and the values in the portal sites also exceeded those in peripheral veins. Of note, the levels in the superior mesenteric vein were comparable to those in both left and right gastric veins.

*Table I. Levels of pro-S and Derived Peptides in Venous Plasma from Regions of the GI Tract in Four Anesthetized, Obese Women*

| Subject | Antecubital vein | Hepatic portal vein | Superior mesenteric vein | Right gastric vein | Left gastric vein |
|---|---|---|---|---|---|
| | Pro-S, S-14/S-13 | | | | |
| | *pg equiv S-14/ml* | | | | |
| 1 | 9 | 20 | 15 | 28 | 73 |
| 2 | 35 | 42 | 45 | 50 | 68 |
| 3 | 23 | 34 | 44 | 51 | 69 |
| 4 | 18 | 34 | 28 | 91 | 43 |
| | S-28 | | | | |
| | *pg/ml* | | | | |
| 1 | 63 | 100 | 93 | 88 | 161 |
| 2 | 24 | 86 | 90 | 88 | 104 |
| 3 | 34 | 56 | 95 | 113 | 95 |
| 4 | 15 | 25 | 36 | 39 | 43 |

In Fig. 7 are displayed the values for the various peptides in peripheral plasma from patients in whom the stomach or pancreas or both had been resected. Basal levels of pro-S, S-14/S-13 (fraction 1) were similar to those found in normal individuals (Fig. 4 *A*). In the two patients with total gastrectomy, there was a small rise in these peptides by 30 min, whereas in the patient with the total gastrectomy and pancreatectomy and the patient with total pancreatectomy, no increase was seen. In all three patients whose stomach had been removed, basal levels of S-28 in fraction 2 were at the upper range or exceeded those seen in healthy men (Fig. 3 *E*). After food intake, there was a twofold increase in S-28 within 30–60 min with a relatively rapid decline in the ensuing 120 min. In the patient with a total pancreatectomy, S-28 levels were elevated from baseline by 60 min ($P < 0.001$), which persisted throughout the following 3 h. The rapid delivery of nutrient to the small intestine in the patients with total gastrectomy was confirmed by markedly increased glucose and insulin levels that peaked within 30–60 min after food intake.

## Discussion

These studies confirm and extend information related to peptides cleaved from pro-S and secreted into the mammalian circulation. We have been able to directly measure S-28 and S-14, and have indirect evidence that S-13 and pro-S are also present. Heretofore, pro-S and the peptides processed at its COOH-terminus have not been easily distinguished because the majority of antisera used for their detection are nonspecific and physical methods for their separation are cumbersome and have not been extensively used. Thus, it is widely held that S-14 is the major circulating peptide (1, 15, 19, 21). Using HPLC, Shoelson et al. (23) reported that S-13 made up 60% of the total somatostatins in three subjects. Rabbani and Patel also found S-13 and S-14 in the urine of normal volunteers (36). With the AS-77 RIA, which is specific for S-14, we have recorded basal plasma levels of this peptide that are much lower than the published values measured as total pro-S related peptides in intact plasma or after their separation by gel filtration. Levels of peptides either in fraction 1 from the F-4 immunoadsorbent or coincident with the peak of authentic S-14 on gel filtration were significantly greater than the values obtained by AS-77 RIA, suggesting that the residual peptide coeluting with S-14 corresponds to S-13. Under the conditions used in our HPLC, we were unable to separate S-14 from S-13 and found only a single peak in fraction 1 from the F-4 immunoadsorbent that coincided with S-14/S-13. Less than 10% of the peak by AS-10 RIA was recorded in the AS-77 RIA. Because we collected blood under conditions minimizing the rapid in vitro hydrolysis of the $Ala^1$ of S-14 by plasma aminopeptidases (37), we contend that, in our subjects, S-13 accounted for up to 80% of the circulating component usually attributed to S-14. It is also possible, but not yet established, that the $Gly^2$ of S-14 is removed resulting in S-12. We have found that S-14 is released intact from D cells within monolayer cultures of the rat islet (unpublished results); hence, if this peptide is also secreted as such from all somatostatin containing cells in man, it seems likely that the $NH_2$-terminal alanine is deleted during the translocation of S-14 across capillary endothelial cells or while traversing organs such as the liver or lung. In this context, Sacks et al. (38), and Ruggere and Patel (39) have shown that the S-14 is rapidly converted to S-13 by the rat liver. The latter investigators have also found that, when perfused through rat liver and lung, S-28 is cleaved sequentially at its $NH_2$-terminus leading to S-25 and eventually to S-14 (39, 40). However, we were unable to show by

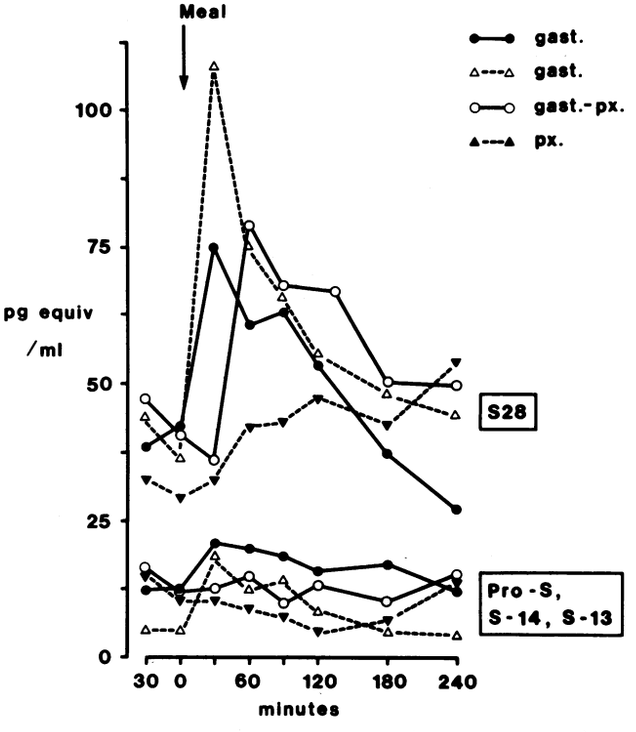


*Figure 7.* Levels of pro-S-derived peptides in plasma with total gastrectomy (gast) and/or pancreatectomy (px) before and after the ingestion of a liquid meal (Ensure Plus, 700 kcal). Peptides corresponding to pro-S, S-14/S-13, and S-28 were measured in fractions 1 and 2 from the F-4 immunoadsorbent by AS-10 RIA as described in legends to Figs. 5 *A* and 6, respectively.

HPLC that S-25, retained by the F-4 immunoadsorbent, was present in human plasma. Moreover, because S-14 did not change after food intake despite increases in S-28, the liver would not appear to be a major source of S-14 through transformation of S-28.

In the basal state, we found that S-28 levels were fourfold greater than those of S-14/S-13. In the dog and man, the $t_{1/2}$ of S-14 ranged between 0.6 and 1.7 min and that for S-28 was 2.8 min (41), which we have confirmed in man (unpublished results). Therefore, when differences in clearance is taken into account, under steady state conditions, the amounts of S-14/S-13 and S-28 delivered into the circulation are probably roughly equivalent.

After food intake in dog and man, increases in SLI have been repeatedly observed (15, 17–19). Few studies, however, have resolved SLI into its components. Using gel filtration, Schusdziarra et al. (15) and Dunning and Taborsky (42), reported that only S-14 increased after feeding dogs a meal of liver in 1 N HCl. Because canine plasma contains S-28 (29), and in our hands, only S-28 increased in dogs fed Ensure (unpublished results), it is possible that acidified liver extract may have led to gastric retention and release of S-14 from the stomach. In man, both S-28 and S-14 have been reported to rise after intake of a mixed meal with most of the increase attributed to S-28 (23, 29, 30). In this study, we have confirmed the rise in S-28 but we were unable to detect any significant changes in S-14/S-13. Although we cannot readily explain these divergent findings, in none of the published reports was there evidence of protection against the actions of aminopeptides, therefore, conversion of S-28 to smaller molecular weight species coinciding with S-14/S-13 by gel filtration may have occurred. It is of interest that pro-S also did not increase after food intake. Because it is plausible that it is cosecreted with S-28, the lack of a perceptible increase may be due to our inability to precisely quantify it lacking a purified standard. Alternatively, increases in the circulating pool from cells in which S-28 was processed may have been obscured by the contribution of pro-S from those cells in which S-14 was generated and unaffected by ingested nutrient.

It is generally agreed that most of the circulating pro-S-related peptides originate in the gastro-entero-pancreatic organs (1, 2). However, the pancreas contributes less than do either stomach or intestine (43, 44) and as the amount of S-28 in rat pancreas and canine stomach are small and unlikely to be intermediates in the processing to S-14 (24, 26), it is probable that neither of these organs are major sources of circulating S-28. This is in keeping with our findings in pancreatectomized patients in whom levels of S-28 and S-14/S-13 were similar to those of normal men during basal and postprandial phases. In this study, S-28 and S-14/S-13 were released from both the stomach and small intestine. Our inability to measure regional blood flow in man does not permit definite conclusions as to the relative amounts of each peptide delivered to the hepatic portal venous drainage. Nonetheless, estimates can be derived from blood flow measurements from various visceral organs in the baboon, which is similar to man. In these animals, the resting blood flow from the small intestine is six- to sevenfold greater than from the stomach (45). Therefore, even though the static measurements of S-28 were the same and, in the case of the S-14/S-13, higher in the veins from the stomach, it is plausible that, during the basal state, most of these peptides originated from cells within the small intestine. The early onset and twofold rise in S-28 levels in patients with total gastrectomy, comparable to the changes over a longer period in normal subjects, imply that the stomach contributes little to the postprandial increase in S-28. These observations thus provide additional support to in vitro data suggesting that S-28 is the terminal peptide processed from pro-S in epithelial cells of the small intestine, whereas S-14 is the final product in most gastric D cells and gastrointestinal neurons (25).

Our findings that S-28 but not S-14 levels are increased after nutrient intake raises the possibility that they serve divergent functions. There is considerable evidence for a paracrine role for S-14 (1, 7, 8). The presence of S-14/S-13 in blood may reflect their spillover from multiple sources rather than the assumption that they are hormones. In contrast, it is reasonable to postulate that S-28 might act on such distant targets as the B cells in the endocrine pancreas. Bonner-Weir and Orci (46) have shown that the central mass of B cells in the islet is perfused centrifugally, thereby making it unlikely that S-14 released from D cells within the mantle could have access to the B cell core. Recently, Samols and Stagner concluded that the cellular sequence of perfusion is from B to A to D cells (47). Combined with our inability to demonstrate postprandial changes in S-14, these findings thus, do not support a primary regulatory role for this peptide on insulin secretion. The notion that S-28 might modulate insulin release is supported by studies indicating that it is not only more potent than S-14 in inhibiting insulin release (48) but is bound to B cells with a higher affinity (49, 50). Proof of a physiologic action for circulating S-14/S-13 or S-28 has been indirect. Infusions of S-14 in man to attain steady-state levels approximating post-prandial increments of SLI result in suppression of several peptides from the pancreas and GI tract (21). Nonetheless, because we have been unable to document a significant change in S-14/S-13 after eating, we contend that such studies are not relevant. By comparison, S-28 infusions have led to inordinately high concentrations and their effects cannot be equated with physiologically appropriate postcibal levels (51). Schusdziarra et al. (20) postulated that the S-14 might play a role in nutrient homeostasis based on enhanced levels of several peptide hormones including insulin in dogs after infusion of antisomatostatin serum. Although they believed that these perturbations reflected removal of circulating S-14/S-13, it is also possible that they were due to neutralization of S-28 levels. To our knowledge, this question has not been resolved by infusion of immunoglobulins against S-28.

In summary, these studies have shown that S-28, but not S-14/S-13, is increased in the peripheral blood of man after ingestion of a mixed nutrient meal. The selective rise in S-28 during food intake from intestinal endocrine cells, implies that this peptide acts as a hormone that may modulate several functions involved in nutrient absorption and assimilation.

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## References

1. Reichlin, S. 1983. Somatostatin. *N. Engl. J. Med.* 309:1495–1501 and 1556–1563.

2. McIntosh, C. H. S. 1985. Gastrointestinal somatostatin: distribution, secretion and physiological significance. *Life Sci.* 37:2043–2058.

3. Patel, Y. C., and S. Reichlin. 1978. Somatostatin in hypothalamus, extrahypothalamic brain, and peripheral tissues of the rat. *Endocrinology.* 102:523–530.

4. Johansson, O., T. Hokfelt, and R. P. Elde. 1984. Immunohistochemical distribution of somatostatin-like immunoreactivity in the central nervous system of the adult rat. *Neuroscience.* 13:265–339.

5. Dubois, M. 1975. Immunoreactive somatostatin is present in discrete cells of the endocrine pancreas. *Proc. Natl. Acad. Sci. USA.* 72:1340–1343.

6. Tange, A. 1983. Distribution of peptide-containing endocrine cells and neurons in the gastrointestinal tract of the dog: immunohistochemical studies using antisera to somatostatin, substance P, vasoactive intestinal polypeptide, met-enkephalin, and neurotensin. *Biomed. Res.* 4:9–24.

7. Samols, E., S. Bonner-Weir, and G. C. Weir. 1986. Intra-islet insulin-glucagon-somatostatin relationships. *Clin. Endocrinol. Metab.* 15:33–59.

8. Makhlouf, G. M. 1987. Antral somatostatin: a paracrine regulator of gastrin secretion. *In* Somatostatin: Basic and Clinical Status. S. Reichlin, editor. Plenum Publishing Corp., New York. 239–251.

9. Kronheim, S., M. Berelowitz, and B. L. Pimstone. 1978. The characterization of somatostatin-like immunoreactivity in human serum. *Diabetes.* 27:523–529.

10. Penman, E., J. A. H. Wass, A. Lund, P. J. Lowry, J. Stewart, A. M. Dawson, G. M. Besser, and L. H. Rees. 1979. Development and validation of a specific radioimmunoassay for somatostatin in human plasma. *Ann. Clin. Biochem.* 16:15–25.

11. Patel, Y. C., T. Wheatley, D. Fitz-Patrick, and G. Brock. 1980. A sensitive radioimmunoassay for immunoreactive somatostatin extracted plasma: measurement and characterization of portal and peripheral plasma in the rat. *Endocrinology.* 107:306–313.

12. Arimura, A. 1980. Radioimmunoassay for somatostatin in tissue and blood. *In* Gastrointestinal Hormones. G. B. Jerzy Glass, editor. Raven Press, New York. 841–852.

13. Hilsted, L., and J. J. Holst. 1982. On the accuracy of radioimmunological determination of somatostatin in plasma. *Reg. Pept.* 4:13–31.

14. Utsumi, M., H. Makimura, K. Ishihara, S. Morita, and S. Baba. 1979. Determination of immunoreactive somatostatin in rat plasma in responses to arginine, glucose and glucagon infusion. *Diabetologia.* 17:319–323.

15. Schusdziarra, V., E. Zyznar, D. Rouiller, V. Harris, and R. H. Unger. 1980. Free somatostatin in the circulation: amounts and molecular sizes of somatostatin-like immunoreactivity in portal, aortic, and vena caval plasma of fasting and meal-stimulated dogs. *Endocrinology.* 107:1572–1576.

16. Rouiller, D., V. Schusdziarra, V. Harris, and R. H. Unger. 1980. Release of pancreatic and gastric somatostatin-like immunoreactivity in response to the octapeptide of cholecystokinin, secretin, gastric inhibitory polypeptide, and gastrin-17 in dogs. *Endocrinology.* 107:524–529.

17. Chayvialle, J. A., M. Miyata, P. L. Rayford, and J. C. Thompson. 1980. Effects of test meal, intragastric nutrients, and intraduodenal bile on plasma concentrations of immunoreactive somatostatin and vasoactive intestinal peptide in dogs. *Gastroenterology.* 79:844–852.

18. Wass, J. A. H., E. Penman, J. R. Dryburgh, D. Tsiolakis, P. L. Goldberg, A. M. Dawson, G. M. Besser, and L. H. Rees. 1980. Circulating somatostatin after food and glucose in man. *Clin. Endocrinol.* 12:569–574.

19. Tsuda, K., H. Sakurai, Y. Seino, S. Seino, K. Tanigawa, H. Kuzuya, and H. Imura. 1981. Somatostatin like immunoreactivity in human peripheral plasma measured by radioimmunoassay following affinity chromatography. *Diabetes.* 30:471–474.

20. Schusdziarra, V., E. Zyznar, D. Rouiller, G. Boden, J. C. Brown, A. Arimura, and R. H. Unger. 1980. Splanchnic somatostatin: a hormonal regulator of nutrient homeostasis. *Science (Wash. DC).* 207:530–532.

21. Zyznar, E. S., A. O. Pietri, V. Harris, and R. H. Unger. 1981. Evidence for the hormonal status of somatostatin in man. *Diabetes.* 30:883–886.

22. Conlon, J. M., M. Bridgeman, and K. G. M. M. Alberti. 1982. The nature of big plasma somatostatin: implications for the measurement of somatostatin-like immunoreactivity in human plasma. *Anal. Biochem.* 125:243–252.

23. Shoelson, S. E., K. S. Polonsky, T. Nakabayashi, J. B. Jaspan, and H. S. Tager. 1986. Circulating forms of somatostatin-like immunoreactivity in human plasma. *Am. J. Physiol.* 250 (*Endocrinol. Metab. 13*):E428–E434.

24. Patel, Y. C. 1983. A high molecular weight form of somatostatin-28 [1-12]-like immunoreactive substance without somatostatin-14 immunoreactivity in the rat pancreas. Evidence that somatostatin-14 synthesis can occur independently of somatostatin-28. *J. Clin. Invest.* 72:2137–2143.

25. Baskin, D. G., and J. W. Ensinck. 1984. Somatostatin in epithelial cells of intestinal mucosa is present primarily as somatostatin-28. *Peptides (NY).* 5:615–621.

26. Chiba, T., J. Park, and T. Yamada. 1988. Biosynthesis of somatostatin in canine D fundic cells. *J. Clin. Invest.* 81:282–287.

27. Patel, Y. C., T. Wheatley, and C. Ning. 1981. Multiple forms of immunoreactive somatostatin: comparison of distribution in neural and non-neural tissues in portal plasma of the rat. *Endocrinology.* 109:1943–1949.

28. Millar, R. P., W. J. Sheward, I. Wegener, and G. Fink. 1983. Somatostatin-28 is a hormonally active peptide secreted into hypophysial portal vessel blood. *Brain Res.* 260:334–337.

29. Shoelson, S., K. Polonsky, H. Docherty, J. Jaspan, and A. Rubenstein. 1982. Evidence for the presence of somatostatin 28 in plasma. *Diabetes.* 31:474–477.

30. Penman, E., P. J. Lowry, J. A. H. Wass, V. Marks, A. M. Dawson, G. M. Besser, and L. H. Rees. 1980. Molecular forms of somatostatin in normal subjects and in patients with pancreatic somatostatinoma. *Clin. Endocrinol.* 12:611–620.

31. Baldissera, F. G. A., M. A. Munoz-Perez, and J. J. Holst. 1983. Somatostatin 1-28 circulates in human plasma. *Reg. Pept.* 6:63–69.

32. Penman, E., J. A. H. Wass, S. Medbak, L. Morgan, J. M. Lewis, G. M. Besser, and L. H. Rees. 1981. Response of circulating immunoreactive somatostatin to nutritional stimuli in normal subjects. *Gastroenterology.* 81:692–699.

33. Polonsky, K. S., S. E. Shoelson, and H. M. Docherty. 1983. Plasma somatostatin 28 increases in response to feeding in man. *J. Clin. Invest.* 71:1514–1518.

34. Aron, D. C., R. C. Andrews, J. E. Dixon, and B. A. Roos. 1984. Identification of cellular prosomatostatin and non-somatostatin peptides derived from its amino terminus. *Biochem. Biophys. Res. Commun.* 124:450–456.

35. Von Schenck, H., I. Larsson, and J. Thorell. 1976. Improved radioiodination of glucagon with the lactoperoxidase method. Influence of pH on iodine substitution. *Clin. Chim. Acta.* 69:225–232.

36. Rabbani, S. N., and Y. C. Patel. 1988. Measurement and characterization of somatostatin-14-like immunoreactivity in human urine. *J. Clin. Endocrinol. & Metab.* 66:1050–1055.

37. Behal, F. J., R. D. Hamilton, F. B. Dawson, and L. C. Terrell. 1964. A study of the activity of human aminopeptidase components on dipeptides and chromogenic substrates. *Arch. Biochem. Biophys.* 108:207–214.

38. Sacks, H. S., L. C. Terry, R. K. Wright, and F. B. Stentz. 1984. Somatostatin metabolism: differences in clearance of N-terminal and central portions of molecule during perfusion of rat liver. *Am. J. Physiol.* 246 (*Gastrointest. Liver Physiol. 9*):G226–G234.

39. Ruggere, M. D., and Y. C. Patel. 1985. Hepatic metabolism of somatostatin-14 and somatostatin-28. Immunochemical characterization of the metabolic fragments and comparison of cleavage sites. *Endocrinology.* 117:88–96.

40. Ruggere, M. D., and Y. C. Patel. 1985. Somatostatin metabolism by the isolated rat lung. *Endocrinology.* 117:1870–1873.

41. Seal, A., T. Yamada, H. Debas, J. Hollenshead, B. Osadchey, G. Aponte, and J. Walsh. 1982. Somatostatin-14 and -28: clearance and potency on gastric function in dogs. *Am. J. Physiol.* 243 (*Gastrointest. Liver Physiol.* 6):G97–G102.

42. Dunning, B. E., and G. J. Taborsky, Jr. 1985. Immunoneutralization reveals endocrine actions of somatostatin on the insulin (IRI) and glucagon (IRG) response to intragastric liver and HCl. *Diabetes.* 34:7a. (Abstr.)

43. Taborsky, J. G., Jr., and J. W. Ensinck. 1984. Contribution of the pancreas to circulating somatostatin-like immunoreactivity in the normal dog. *J. Clin. Invest.* 73:216–223.

44. Marre, M., J. Miller, A. M. Helman, and R. Assan. 1983. Reciprocal gastropancreatic modulations for the release of somatostatin-like immunoreactivity, glucagon and insulin in the rat. *Diabetes.* 32:768–773.

45. Hales, J. R. S., L. B. Rowell, and R. B. King. 1979. Regional distribution of blood flow in awake heat-stressed baboons. *Am. J. Physiol.* 237:H705–H712.

46. Bonner-Weir, S., and L. Orci. 1982. New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes.* 31:883–889.

47. Samols, E., and J. Stagner. 1988. The cellular sequence of perfusion in the rat islet appears to be B. A. D. *Clin. Res.* 36:588*a*. (Abstr.)

48. Mandarino, L., D. Stenner, W. Blanchard, S. Nissen, J. Gerich, N. Ling, P. Brazeau, P. Bohlen, F. Esch, and R. Guillemin. 1981. Selective effects of somatostatin-14, -25, and -28 on in vitro insulin and glucagon secretion. *Nature (Lond.).* 291:76–77.

49. Reubi, J. C., J. Rivier, M. Perrin, M. Brown, and W. Vale. 1982. Specific high affinity binding sites for somatostatin-28 on pancreatic B cells: differences with brain somatostatin receptors. *Endocrinology.* 110:1049–1051.

50. Amherdt, M., Y. C. Patel, and L. Orci. 1987. Selective binding of somatostatin-14 and somatostatin-28 to islet cells revealed by quantitative electron microscopic autoradiography. *J. Clin. Invest.* 80:1455–1458.

51. Konturek, S. J., N. Kwiecien, W. Obtulowicz, W. Bielanski, J. Olefsky, and A. V. Schally. 1985. Effects of somatostatin-14 and somatostatin-28 on plasma hormonal and gastric secretory responses to cephalic and gastrointestinal stimulation in man. *Scand. J. Gastroenterol.* 20:31–38.