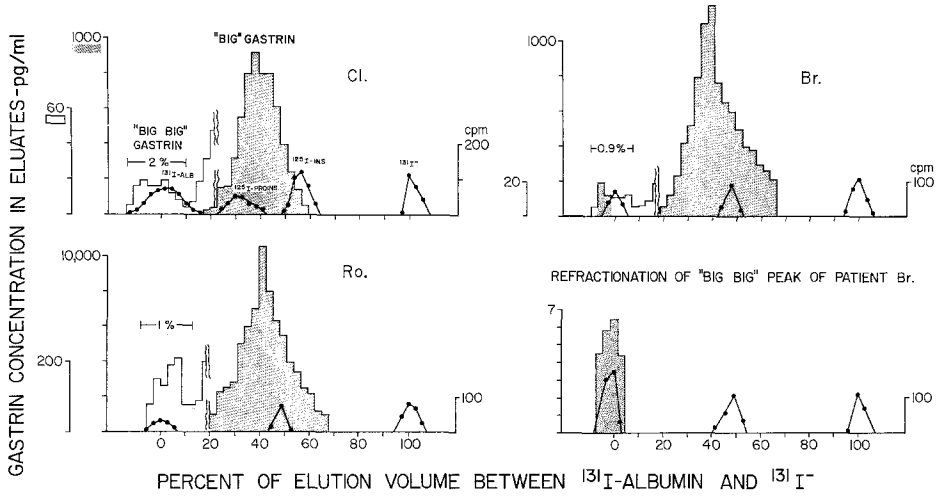

Fig. 1 - Distribution of immunoreactive gastrin in plasma of three patients with Zollinger-Ellison syndrome. Gastrin concentration in the plasmas and the volume applied to the columns were: Cl 3 ng/ml, 2.0 ml, Ro 120 ng/ml, 0.2 ml and Br 5 ng/ml, 2.0 ml. The concentrations in the early eluates are shown as white and on the expanded scale. The concentration of labeled marker molecules are shown in this and succeeding figures in solid circles. (Lower right) Refractionation of "big big" peak (stippled tubes) from patient Br shown in upper right.

with Zollinger-Ellison syndrome, were fortified with ^{125}I- or ^{131}I-labeled marker molecules (albumin, proinsulin, insulin and iodide), applied to the columns and eluted with the same albumin - barbital buffer used to equilibrate the columns. These markers were used since "big" gastrin with a molecular weight of about 7000 emerges between proinsulin and insulin and heptadecapeptide gastrin with a molecular weight of 2100 emerges after insulin on Sephadex gel filtration (6, 7, 8). Flow rates were between 10 and 20 ml per hour and 1 ml fractions were collected for assay. Gastrin concentrations in the various fractions were determined by a method of radioimmunoassay previously described (12) using an antiserum with a sensitivity of 0.2 pg porcine gastrin I per ml. Eluates containing the larger molecular weight fraction were combined, incubated without or with trypsin (1 mg/ml) at 37°C for 20 minutes, boiled for 3 minutes to destroy the trypsin activity, and fractionated again on Sephadex G 50 columns.

AND NOW, "BIG, BIG" GASTRIN

Rosalyn S. Yalow and Solomon A. Berson*

Nuclear Medicine Service, Veterans Administration Hospital, Bronx, N. Y. 10468
and Department of Medicine, Mt. Sinai School of Medicine of CUNY, N. Y. 10029

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SUMMARY: Plasmas from patients with Zollinger-Ellison syndrome and extracts from the jejunum obtained post-mortem contain a minor component of immunoreactive gastrin of considerably greater size than heptadecapeptide gastrin and the previously described "big" gastrin. This component maintains its integrity on refractionation but releases an immunoreactive heptadecapeptide-like gastrin following tryptic digestion.

Recognition of the multiple forms of parathyroid hormone (1), insulin (2, 3), glucagon (4), growth hormone (5), gastrin (6, 7, 8) and ACTH (9) in plasma and tissues of origin has promoted further search for hitherto unsuspected hormonal components that differ from the well-recognized form of the hormone. Evidence was recently presented that in addition to proinsulin there exists a "big, big" insulin, with a molecular weight greater than albumin. It was found to be the major component of immunoreactive insulin in the plasma of an insulinoma suspect and a minor component in extracts from insulinomas and normal pancreas (10, 11). In the present study, we demonstrate that in plasmas from patients with Zollinger-Ellison syndrome and in jejunal extracts there is a minor component of immunoreactive gastrin of considerably larger size than the previously described "big" gastrin (6, 7, 8).

METHODS

Columns of Sephadex G 50, fine, 1 cm x 50 cm, were equilibrated with barbital buffer, 0.02 M, pH 8.6, containing 0.25% human serum albumin. Post-mortem material from the gastrointestinal tract was obtained within 6 hours after death in 3 cases; mucosa from the antrum and proximal jejunum were stripped and frozen. Gastrin was extracted by boiling in water for 30 minutes. Following centrifugation the supernatants were stored frozen until fractionation. These extracts and plasmas from three other patients,

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RESULTS

On gel filtration, recoveries ranged from 70% to 130% of immunoreactive gastrin placed on the column. From 0.9 - 2% of the total gastrin immunoreactivity in the plasmas from the three patients with Zollinger-Ellison syndrome was found in the eluates emerging from the Sephadex filtration in the albumin region (Fig. 1). In calibration runs on these columns ^{125}I-hGH elutes at about 16% of the elution volume between ^{131}I-albumin and ^{131}I$^-$. The new immunoreactive gastrin component is therefore distinctly larger than hGH and quite close to albumin in molecular weight. This component is designated as "big, big" gastrin. On refractionation, the "big, big" peaks were eluted in the same region, as is illustrated in Fig. 1 for patient Br for whom the peak was least definite at the initial elution.

After treatment with chymotrypsin-free trypsin (1 mg/ml) for 20 minutes at 37°C, the "big, big" peak had diminished to about 15% of its initial amount and virtually all of the decrement could be accounted for by conversion to a heptadecapeptide-like component (Fig. 2). The control sample without trypsin incubated side-by-side with the trypsin-treated sample showed no detectable change during the same time (Fig. 2).

Antral extracts did not contain significant "big, big" gastrin components. However, in jejunal extracts obtained from the post-mortem specimens the fraction of immunoreactive gastrin in the "big, big" region ranged from 6% to 24% (Fig. 3). On refractionation on Sephadex G 50, the "big, big" peaks maintained their integrity.

DISCUSSION

The present study demonstrates that both in plasma and in extracts of proximal jejunum, there exists a minor component of immunoreactive gastrin close to albumin in molecular weight which does not convert to another form on refractionation. Like "big" gastrin (8), the fraction of immunoreactivity in the "big, big" form increases distally in descending the gastrointestinal tract, being virtually undetectable in the antrum and amounting to 6-24% of

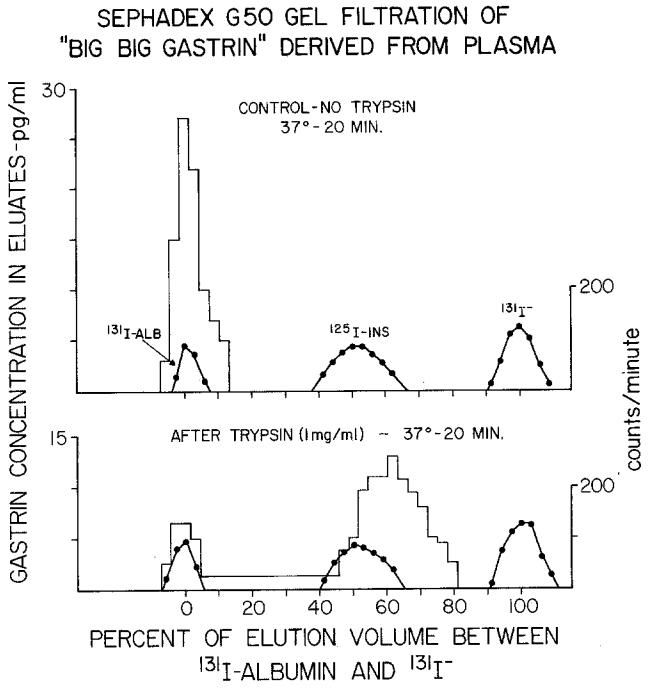

Fig. 2 - Refractionation of "big big" peak on Sephadex G 50 following incubation at 37°C without (top) and with (bottom) trypsin (1 mg/ml).

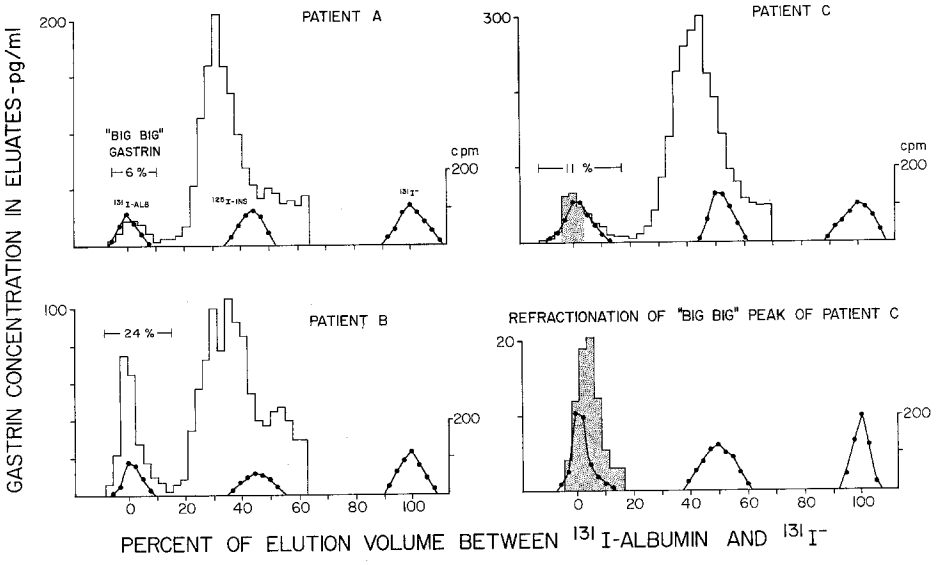

Fig. 3 - Distribution of immunoreactive gastrin in extracts of the proximal jejunum obtained from three patients post-mortem. Immunoreactive gastrin in the extracts ranged from 4 to 10 ng/g mucosa and volumes containing 1 to 3 ng were applied to the columns. (Lower right) Refractionation of "big big" peak (stippled tubes) from patient C shown in upper right.

the gastrin immunoreactivity in the proximal jejunum. Following tryptic digestion of the "big, big" peak from plasma no "big" gastrin but only heptadecapeptide-like gastrin was detected. Since "big" gastrin is converted virtually instantly to heptadecapeptide-like gastrin on treatment with trypsin (7, 10), this does not preclude the possibility that "big" gastrin is contained within the "big, big" gastrin. The failure to convert "big, big" gastrin to a smaller form except by tryptic digestion suggests that the "big, big" component is not a polymerized form of the smaller gastrins but contains within it in peptide linkage at least heptadecapeptide gastrin, if not "big" gastrin. Since trypsin splits peptide bonds whose carboxyl group is donated by a basic amino acid, lysine or arginine, it appears likely that "big, big" gastrin is composed of smaller gastrin linked at its amino terminal to a basic amino acid of a larger peptide.

The results from a number of studies (7, 9-11, 13) now suggest that for several hormones the usual, well-recognized form can be derived from a larger molecular weight form by treatment with trypsin.

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