

**RADIOIMMUNOASSAY OF GASTRIN IN HUMAN SERUM****USING ANTISERUM AGAINST PENTAGASTRIN**

J. D. Young, D. J. Byrnes, D. J. Chisholm, F. B. Griffiths and L. Lazarus

*Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, Australia*

Radioimmunoassay systems for gastrin have been described recently (1,2), and one of the authors, working on the hypothesis that antibodies may be formed against small peptide subunits, described production of antibodies to the C-terminal tetrapeptide amide of gastrin (3). This paper describes the similar development of antiserum to the gastrin-like pentapeptide Pentagastrin, (Imperial Chemical Industries U.K.) I.C.I.50, 123, -t-butylloxycarbonyl- $\beta$  Ala.Try.Met.Asp.Phe.NH<sub>2</sub><sup>-</sup>, and shows that this antiserum may be used satisfactorily for the measurement of gastrin in human serum.

**METHODS**

Synthetic human gastrin-1 (hG1) was labeled with <sup>131</sup>I by the method of Hunter and Greenwood (4). The labeled gastrin was then purified by gel filtration on a Sephadex-G 10 column. Using 5mCi of <sup>131</sup>I (Amersham IBS-3) and 5  $\mu$ g of hG1, a specific activity of 200–500 mCi/mg was obtained.

Antiserum was raised in chickens because of their dual features of low immune tolerance (5,6) and small gastric acid secretory capacity. Pentagastrin (40 mg) was conjugated to rabbit serum albumin (100 mg) using carbodiimide (7). The conjugate mixture was then freeze-dried, and 4 mg of the powder in 0.25 ml of saline was emulsified with 0.25 ml of Freund's complete adjuvant and injected into the hindquarter of two white Leghorn chickens every alternate week for 5 months. After this period of immunization, serum obtained from the chickens failed to show significant binding of <sup>131</sup>I-hG1. To accentuate the immune response, two further injections of conjugated Pentagastrin were administered concurrently with i.m. injections of  $2 \times 10^{10}$  organisms of H-pertussis vaccine (CSL) over a 5-day period (5,6). Serum obtained 1 week later showed antibody binding of <sup>131</sup>I-hG1 up to a dilution of 1 in 5,000.

In the radioimmunoassay system Amberlite CG400 Type 11 hydroxide cycle anion exchange resin was used to separate the "free" from the antibody-bound labeled hormone (8). Optimum sen-

sitivity was achieved using a 1-in-500 dilution of antiserum. All dilutions in the assay system were made with Sorenson's phosphate buffer (pH 7.5, 0.15 M) containing 0.5% bovine serum albumin and 0.005% mercuric chloride. Standard concentrations of hG1 (0.1–5 ng) were freshly prepared in 0.5 ml of 0.5% BSA on the day of assay and then mixed in 0.5 ml of 3.75% BSA on the day of assay and then mixed in 0.5 ml of 3.75% gelatine to simulate the protein content of human serum (9). The volume of samples other than standards were adjusted using 0.5 ml of 0.5% BSA. Laboratory procedure for the assay consisted of the addition of 0.5 ml of unknown serum (or standard) to a 20-ml glass scintillation vial with 0.5 ml of appropriately diluted antiserum. This mixture was then allowed to stand at 20°C for 2 hr after which 10 pg of <sup>131</sup>I-hG1 in 4 ml of buffer were added with an automatic pipetting unit. A further incubation of 14 hr at 20°C was followed by the addition of 200 mg of ion exchange resin after which the vials were shaken in a fast swing arm shaker at 180 oscillations/min for 2 hr at 20°C. The resin, which takes up the free hormone, was then washed with 50 ml of distilled water and the radioactivity bound to the resin was measured in a well-type gamma scintillation counter, the radioactivity being expressed as a percentage of the added <sup>131</sup>I tracer (% resin uptake). From the resin uptake of the standards the concentration of gastrin in the unknown serum was determined graphically.

**DISCUSSION**

The specificity of the assay system was tested by the addition of either 1 mg of porcine insulin, 1 mg of bovine-porcine glucagon or 100  $\mu$ g of synthetic secretin. No elevation of resin uptake was recorded, indicating that there was no cross reactivity of these hormones with the antiserum at the levels tested.

Received July 7, 1969; revision accepted Sept. 5, 1969.

For reprints contact: John D. Young, St. Vincent's Hospital, Garvan Institute of Medical Research, Darlinghurst, N.S.W., Sydney 2010, Australia.

The addition of hG1, Pentagastrin and highly purified pancreozymin-cholecystokinin (PZ-CCK, Jorpes) did, however, increase the resin uptake as did serum from a patient with Zollinger-Ellison syndrome. When dose-response curves were plotted semi-logarithmically against percent resin uptake, a variable response was obtained (Fig. 1). It can be seen that the responses are linear and that the serially diluted serum is parallel to that of hG1 but that the response for Pentagastrin is not parallel. The difference in the Pentagastrin slope is probably due to antibody binding against the alanyl group of Pentagastrin which is absent from the parent hG1 molecule.

Cross reactivity was noted with PZ-CCK as was anticipated on the basis of the identical C-terminal tetrapeptide of hG1 and PZ-CCK, and the dose response curve for PZ-CCK was also parallel to that of gastrin. It can be seen from Fig. 2 that 1,000  $\mu$ U PZ-CCK was immunologically equivalent to 0.05 ng hG1. This immunological relationship is equimolar and in agreement with the findings of McGuigan (3).

Sensitivity of this assay system was better than 0.1 ng/ml and within assay standard deviation calculated from multiple assays of post-prandial serum was 0.05 ng/ml where mean gastrin content was 1.8 ng/ml.

Preliminary studies using this assay system have shown a mean fasting gastrin level of 0.4 ng/ml ( $\pm 0.3$  s.d.) in 20 normal male subjects. Following oral ingestion of protein, serum levels showed a mean elevation of 2.7 ng/ml (range 2.3 to 3.1 ng/ml). A fasting serum level of 200 ng/ml was recorded in a patient with proven Zollinger-Ellison syndrome.

The significance of the cross reactivity of hG1 and PZ-CCK was studied by simultaneous radioimmunoassay of gastrin and PZ-CCK in serum (10). These studies showed that serum PZ-CCK levels after glucose loads (50–200 G) or steak meals (120 G) did not exceed 1,500  $\mu$ U/ml and that levels of up to 2,400  $\mu$ U/ml may be observed after the ingestion of 60 G of fat or a mixed protein-fat-carbohydrate meal. This latter maximal PZ-CCK response would be immunologically equivalent to 0.12 ng/ml of gastrin (Fig. 2). The gastrin and PZ-CCK response in a normal subject to a fat meal is shown in Fig. 3. It will be noted that at the peak of the PZ-CCK response (which is the highest level recorded in four subjects after a fat meal), the serum gastrin had already fallen below the fasting level, illustrating that at this time the maximal interference with the serum "gastrin" level by PZ-CCK would be less than 0.3 ng/ml.

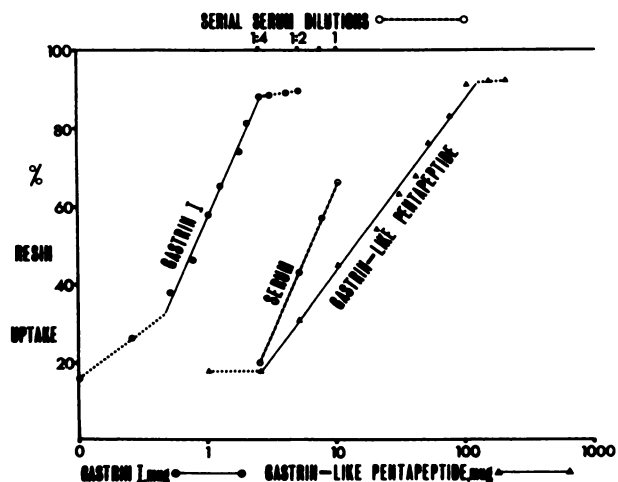


FIG. 1. Typical dose-response relationship between synthetic human gastrin-1, Pentagastrin and serially diluted serum.

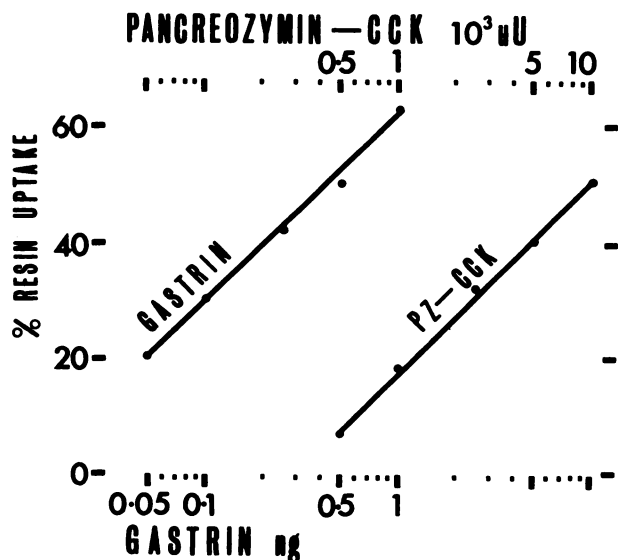


FIG. 2. Dose response relationship between pancreozymin-cholecystokinin and human gastrin-1.

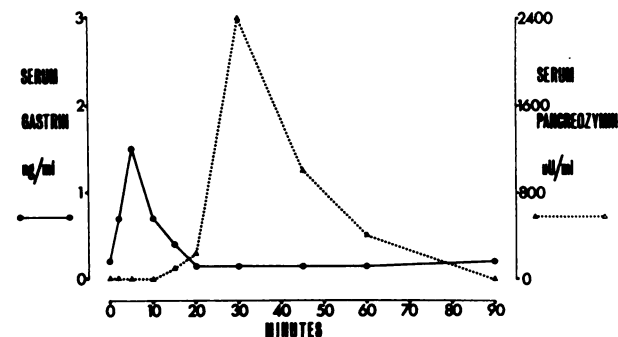


FIG. 3. Simultaneous radioimmunoassay for gastrin and pancreozymin-cholecystokinin following fat meal.

In summary, evidence has been presented that antiserum prepared against the synthetic gastrin-like pentapeptide, Pentagastrin, may be used to set up a radioimmunoassay for gastrin. Although cross reactivity with pancreozymin has been shown and must lead to cautious interpretation of results, it appears that interference with gastrin estimation is only likely when there is a strong stimulus to pancreozymin release or where there is a possibility of multiple hormonal secretion by a gastrin-producing tumor.

#### ACKNOWLEDGMENTS

We acknowledge the gifts of Pentagastrin from I. Proctor, Medical Director, I.C.I.A.N.Z., Melbourne, Australia and purified pancreozymin from E. Jorpes, Karolinska Institute, Stockholm, Sweden.

#### REFERENCES

1. MCGUIGAN, J. E.: Immunochemical studies with synthetic human gastrin. *Gastroenterology* 54:1,005, 1968.
2. ODELL, W. D., CHARTERS, W. D., DAVIDSON, W. D. AND THOMPSON, J. C.: Radioimmunoassay for human gastrin using unconjugated gastrin as an antigen. *J. Clin. Endocrinol. Metab.* 28:1,840, 1968.
3. MCGUIGAN, J. E.: Antibodies to the C-terminal tetrapeptide amide of gastrin. *Gastroenterology* 54:1,012, 1968.
4. HUNTER, W. M. AND GREENWOOD, F. S.: Iodine<sup>125</sup> labelled growth hormone of high specific activity. *Nature* 194:495, 1962.
5. SCHMIDT, M. AND WOLFE, H. R.: Precipitin production in chickens IX. A quantitative study of the antibody response to nine different purified substances. *J. Immunology* 71: 214, 1953.
6. AMBRAMOFF: Precipitin production in chickens XIII. A quantitative study of the effects of simultaneous injection of two antigens. *J. Immunology* 77:94, 1956.
7. GOODFRIEND, T. L., LEVINE, L. AND FASMAN, G. D.: Antibodies to bradykinin and angiotensin. A use of carbo-diimides in immunology. *Science* 144:1,344, 1964.
8. LAZARUS, L. AND YOUNG, J. D.: Radioimmunoassay of human growth hormone using ion exchange resin. *J. Clin. Endocrinol. Metab.* 26:213, 1966.
9. YOUNG, J. D. AND KRAEGEN, E. W.: Simultaneous assay of insulin and glucagon in serum. *Australian J. Exp. Biol. Med. Sci.* 46:697, 1968.
10. YOUNG, J. D., LAZARUS, L., CHISHOLM, D. J. AND ATKINSON, F. F. V.: Radioimmunoassay of pancreozymin-cholecystokinin in human serum. *J. Nucl. Med.* Submitted for publication.

## FIRST CALL FOR NUCLEAR MEDICINE "ART" EXHIBITS FOR 17TH ANNUAL MEETING IN WASHINGTON, D.C.

July 6-12, 1970

The Scientific Exhibits Committee is planning a nuclear medicine art exhibit open only to technicians (technical affiliates and associate members) who will display their best "works of art."\* This "art" may consist of normal and abnormal scans, scintophotos, renograms or other dynamic studies, etc.

All exhibits will be illuminated by available room light. There will be no provisions for transillumination, e.g. view boxes. Photographic prints or Polaroid film (black and white or color), any size, should be mounted on poster board not exceeding 30 in. X 30 in. No more than two boards may be entered for a subject. Exhibits should be clearly titled. Technical information related to the study displayed should be concise yet sufficiently detailed to instruct and assure duplication. Clinical information should be limited to details pertinent to the study. Technician's name and institutional address should appear at lower left corner. Prizes for the best exhibits will be awarded at the annual business meeting. The art will be judged on the basis of quality, presentation, originality and technical detail. Notice of intent to exhibit should be sent before May 1, 1970 to:

GARY A. WOOD  
P.O. Box 479  
Friendswood, Texas 77546

\* "Art" is defined as products of the nuclear medicine professional effort as distinguished from sculpture, painting or photography.