Progress report

On antibodies to gastrin: concerning their production, behavioural characteristics, and uses

Despite early disappointment with attempts to produce antibodies to gastrin by immunizing animals with stringently purified porcine gastrin, experimental methods have been evolved to elicit successfully antibodies to gastrin using several species of laboratory animal, including rabbits, guinea pigs, chickens, and goats¹⁻¹³. Various antigenic materials have been used: these have included crude, incompletely purified porcine gastrin preparations, negatively charged human gastrin I attached to positively charged polyacrylate particles, and the carboxyl-terminal tetrapeptide amide, pentagastrin, or human gastrin I (residues 1-17 or 2-17) covalently conjugated to carrier macromolecules.

Waddell and his associates were the first investigators successfully to produce antibodies to gastrin^{14,15}. They immunized rabbits with a crude gastrin-containing preparation obtained from hog antral mucosa. These studies were actually performed before Gregory and Tracy¹⁶ so elegantly elucidated the structure of porcine gastrin and its definition as a 17-member polypeptide hormone. Waddell and his associates^{14,15} were able to inhibit the capacity of antral mucosal and Zollinger-Ellison tumour tissue extracts to stimulate acid secretion in canine gastric pouches by prior incubation of these extracts with serum obtained from rabbits injected with this crude porcine gastrin preparation. It is virtually certain that inhibition of acid secretion under these conditions was due to the effects of rabbit antibodies to gastrin elicited in response to immunization. These workers were handicapped by the lack of an available pure preparation of gastrin at that time in their attempts to demonstrate the presence of antibodies to gastrin by independent means. Although by immunodiffusion studies they were able to show that rabbit antibodies to a variety of porcine antral mucosal constituents had been produced their data did not convincingly demonstrate, or even strongly suggest, that any of these immunoprecipitin bands represented interaction between gastrin molecules and antibodies to gastrin.

The quest for antibodies to gastrin has been stimulated by the recognition that such antibodies might prove useful in the sensitive measurement of gastrin, by radioimmunoassay, and in the precise localization of gastrin molecules, as for instance, by immunofluorescence²⁴. Fortunately, both of these optimistic anticipations have proven feasible and have been achieved.

Antibodies thus far characterized which react with gastrin molecules may be classified into two general groups: (1) antibodies to intact, or virtually intact, gastrin, and (2) antibodies to portions of the gastrin molecule, eg, those to the gastrin carboxyl-terminal tetrapeptide amide.

One of the most fascinating aspects of the gastrin structure-function story, and certainly one most appropriate when considering the operational characteristics of antibodies to gastrin, relates to considerations of the carboxyl-terminal portion of the gastrin molecule. The carboxy-terminal tetrapeptide amide of gastrin has been clearly demonstrated to contain all of the physiological activities of intact gastrin molecules, thereby representing or containing the functionally active site of the gastrin molecule. A parallel and most enticing observation is that of Mutt and Jorpes¹⁷ who found that the gastrin carboxyl-terminal pentapeptide amide (which, of course, contains the physiologically active tetrapeptide amide) is identical to that contained

in the polypeptide hormone cholecystokinin-pancreozymin. These observations have provided superb opportunities for investigating structure-function relationships as well as considerations of site and effector mechanisms. As might be anticipated and predicted, in all studies of antibodies to gastrin immunological cross reactivity can be demonstrated between these antibodies and cholecystokinin-pancreozymin. Antibodies evoked in response to the gastrin tetrapeptide amide as the antigenic determinant bind cholecystokinin-pancreozymin molecules approximately as well as they do gastrin molecules¹⁸. However, antibodies to intact, or virtually intact, gastrin molecules exhibit minimal binding of cholecystokinin-pancreozymin, when compared with their binding of gastrin molecules, even from other species. Antibodies produced by immunization with intact gastrin or virtually intact gastrin molecules can be readily shown to bind the carboxyl-terminal tetrapeptide amide of gastrin. Antibodies produced utilizing an assortment of techniques exhibit more apparent antibody specificity for, and reactivity with, the smaller tetrapeptide amide portion of the gastrin molecule than the larger remaining portion of the gastrin molecule which contains the first 13 amino acid residues of the peptide^{6, 7, 10}.

The recent availability of antibodies to gastrin and their application to measurement of gastrin by radioimmunoassay have introduced a new glossary of concepts and terms for consideration by the individual whose major interests are in gastroenterology. Some examples include the weight units used to indicate the sensitivity of the radioimmunoassay method being described or to record the amount of gastrin contained in a tissue or fluid. Radioimmunoassay techniques can be so exquisitely sensitive and the quantities in tissues and fluids so small, relative to our usual considerations of proteins, enzymes, etc, that some comments on the weight units used may be of some value. Gastrin concentration, measured by radioimmunoassay in serum or plasma, is commonly expressed as picograms per millilitre (pg/ml). One picogram is equal to 10^{-12} grams, whereas one nanogram (ng) is 10^{-9} grams (or 1,000 times as great in weight as one picogram). Some investigators use the terms millimicrogram (m μ g) and micromicrogram ($\mu\mu$ g) for the nanogram and picogram respectively. More current and more widely accepted usage is that of picogram and nanogram in preference to micromicrogram and millimicrogram. It is my personal view and my suggestion that investigators measuring gastrin, as well as other gastrointestinal hormones, use the terms picogram and nanogram, designations which in the near future will become as familiar to the clinical gastroenterologist as have concentrations of enzyme units, milliequivalents per litre or mg%.

Although a variety of methods have been successful in eliciting detectable antibodies to gastrin, investigators have not had uniform success in the degree of sensitivity which they have achieved in their radioimmunoassay techniques. Level of sensitivity is of obvious importance, inasmuch as some described antibodies to gastrin are insufficiently sensitive to be of use in measuring serum or plasma gastrin levels, unless massively elevated, and are therefore of limited usefulness both clinically and in basic investigative studies. Sensitivities of various radioimmunoassay methods have ranged from 1,000* or 2,000 pg¹² to 1 pg which is the most sensitive radioimmunoassay method for gastrin thus far described¹³. For the benefit of one reading about radioimmunoassay methods for gastrin it is of value to point out that different investigators may choose to use different approaches to describe the sensitivity of their gastrin radioimmunoassay method. Some may indicate that the maximum sensitivity is 'x picograms', referring to the smallest amount of gastrin which can be detected and measured in the immune reaction tube. Others may refer to the maximum sensitivity as 'picograms per ml'. This designation requires clarification, inasmuch as for some this reference is to picograms per millilitre of serum or plasma being analysed for gastrin

whereas, for others, the reference is to picograms per millilitre contained in the entire incubation mixture. As an illustration, the same gastrin radioimmunoassay may be described as having as its maximum sensitivity the capacity to measure 1 pg of gastrin; if that picogram of gastrin is contained in 0.2 ml plasma its maximum sensitivity might be expressed as 5 pg/ml (plasma); if radioimmunoassay were performed in an incubation volume of 2.5 ml sensitivity might be expressed as 0.4 pg/ml, ie, of immune incubation mixture. Thus the greater than 10-fold difference between 0.4 and 5 pg/ml is not a difference in sensitivity but rather a difference in method of expression. One must read carefully, and authors must express their data precisely if the real sensitivity of a radioimmunoassay method is to be coherently stated and understood.

For all antibodies to gastrin, which have been thus far characterized, there is real, although fortunately minimal, immunological cross reactivity with cholecystokinin-pancreozymin molecules. The degree of cross-reactivity of antibodies to gastrin with cholecystokinin-pancreozymin is usually determined by the assessment of the capacity of cholecystokinin-pancreozymin to inhibit the binding of radioiodinated gastrin by antibodies elicited in response to immunization with various gastrin preparations. This interference with antibody binding of radiolabelled gastrin by cholecystokinin-pancreozymin has been compared with varying concentrations of gastrin molecules (and obviously, for maximum validity, compared on an equimolar basis). Various numerical designations have been applied in attempts to describe quantitatively the degree of immunological cross reactivity of antibodies to gastrin with cholecystokinin-pancreozymin. Inasmuch as the slopes of the calibration curves expressing inhibition of antibody binding of radiolabelled gastrin by gastrin or by cholecystokinin-pancreozymin are different, that for cholecystokinin-pancreozymin being more gradual, numerical designations of relative effectiveness of these antibody preparations vary with the positions selected on those curves at which comparisons are made: for example, a much greater apparent cross reactivity of cholecystokinin-pancreozymin with antibodies to gastrin can be detected when lower concentrations of gastrin and cholecystokinin-pancreozymin molecules are compared, whereas at high inhibitory concentrations immunological cross reactivity may appear to be much less, suggesting greater immunological specificity.

All currently available radioimmunoassay techniques depend upon the use of an appropriate method for separating antibody-bound from antibody-free radiolabelled hormone, in this instance the polypeptide hormone gastrin. Among others, the following methods have been successfully applied to separate antibody-bound from free gastrin. Sephadex gel filtration has been used to separate antibody-bound from antibody-free radiolabelled gastrin (or gastrin tetrapeptide) on the basis of differences in molecular size^{1,10}. A 'double antibody technique' has been used to precipitate radiolabelled gastrin which is bound by rabbit antibodies to gastrin by the addition of precipitating goat antibodies to rabbit gamma globulin^{3,6}. Antibody-bound radioiodinated gastrin has been precipitated using ethanol, leaving non-protein-bound gastrin in the supernatant solution^{4,8}. Gastrin, like many other small organic molecules, can be readily adsorbed to the surface of particulate dextran- or albumin-coated charcoal particles whereas antibody-bound gastrin remains free in solution^{5,9}. Anion-binding resins can be used to bind the negatively charged gastrin molecule, separating it from antibody-bound gastrin¹⁰.

It is apparent that many questions, important in clinical gastroenterology and in fundamental aspects of gastrointestinal physiology, may be approached by use of a well characterized, sensitive, and specific radioimmunoassay method for measurement of gastrin. The application of antibodies to gastrin

by radioimmunoassay has confirmed the suspicion that the circulating secretagogue in the Zollinger-Ellison syndrome was indeed the polypeptide hormone gastrin. Hypergastrinaemia, when sought, has been repeatedly demonstrated by radioimmunoassay in patients with the Zollinger-Ellison syndrome^{6,9,10,19}. But, what about patients with peptic ulcer disease? Experimental evidence to date by radioimmunoassay with antibodies to gastrin does not indicate fasting hypergastrinaemia in common varieties of peptic ulcer disease, eg, with gastric or duodenal ulcers of the non-Zollinger-Ellison variety^{9,20,21}. Most provocative information has been acquired from studies using antibodies to gastrin to measure serum and plasma gastrin concentrations in patients with pernicious anaemia, which by virtue of gastric mucosal atrophy and achlorhydria, represents the gastric acid secretory antithesis of the Zollinger-Ellison syndrome: patients with pernicious anaemia have raised serum gastrin levels when compared with control subjects^{9,10,20,22}. Serum gastrin levels of many patients with pernicious anaemia are as markedly raised as are those with the Zollinger-Ellison syndrome. The explanation for fasting hypergastrinaemia in patients with pernicious anaemia appears to reflect the low hydrogen ion concentration of their gastric intraluminal contents. This high pH environment permits release of gastrin, whereas gastrin release is effectively inhibited by acidification of the antrum (beginning inhibition at pH 3.0 and maximum inhibition at pH 1.5)²³. The gastric mucosal atrophic lesion in pernicious anaemia involves principally the body of the stomach with sparing of the antral mucosa in which gastrin-containing cells reside. Thus with oxyntic (parietal) cell failure gastrin release continues without inhibition from gastrin-containing cells secluded safely in the sanctuary of the antrum. Further support for the hypothesis that in pernicious anaemia high gastrin levels result from the withdrawal of acid inhibition of gastrin release is offered by demonstrated reductions in plasma gastrin levels in pernicious anaemia patients after the ingestion of 0.1N HCl¹⁰.

Many questions remain concerning the parts played by gastrin in normal physiology and in assorted gastrointestinal disease states. Antibodies to gastrin greatly enhance our capacities to answer these questions, to probe their significance, to see with the illumination of these answers further questions and meaningful directions for our investigative efforts.

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