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Immunological Studies of Gastrin

Abstract. Hog gastrin prepared by the Gregory method stimulates antibody production in rabbits. Rabbit anti-hog gastrin serum which was reacted with hog, dog, and human gastrins in vitro caused reduction of their gastric stimulatory effect, indicating a biological cross reaction among these species.

The existence of the antral hormone gastrin was postulated by Edkins (*1*) in 1906, but Komarov (*2*) was the first to obtain an active substance from the protein fraction of antral mucosal extracts. Subsequently, other workers (*3, 4*) have obtained substances capable of stimulating gastric secretion from the protein fraction of mucosal extracts prepared by various methods.

We have produced rabbit antibodies to hog gastrin which inhibited the gastric secretory effect of hog, dog, and human gastrin.

Gastrin was prepared from hog antral mucosa by the method of Gregory and Tracy (*4*). The final extract was lyophilized, and approximately 1.0 g of the dry powder was obtained from 1 kg of mucosa. In addition to gastrin, this product contains a quantity of inert protein and inorganic salts. It promotes a flow of acid gastric juice when given by subcutaneous injection to dogs with a gastric fistula or Heidenhain pouch.

New Zealand rabbits (weight, approximately 2.5 kg each) were injected in the four foot pads and the lateral thoracic wall skin with 30 mg of hog gastrin incorporated in complete Freund's adjuvant. Four to six weeks later each rabbit received a booster injection (20 mg of gastrin dissolved in distilled water) subcutaneously in the interscapular region. One week later animals were bled by sterile intracardiac puncture, and the serum was harvested. With merthiolate added as a preservative, the serum was stored at −20°C until used. The anti-hog gastrin titer of this serum was determined by the qualitative preciptin method of Heidelberger and Kendall (*5*); the average titer was 1/5000 to 1/10,000. Higher titers could be obtained with subsequent booster injections.

Agar gel diffusion experiments were performed according to the method of Wilson and Pringle (*6*). Plates were prepared, allowed to stand in a moist chamber at 37°C for 7 days, and then read.

Biological inhibition tests were performed in the following manner. Solutions of hog, dog, and human gastrin preparations were mixed with rabbit antiserum to hog gastrin and allowed to stand overnight in the refrigerator. The mixtures were then injected subcutaneously into dogs with a Heidenhain pouch, and the secretory responses were compared with those obtained by injecting the same doses of gastrin alone. The volumes of solution injected were equal in all cases. A control injection of gastrin reacted with normal rabbit serum (Difco Laboratories) was included in all inhibition experiments.

Table 1. Data of secretory inhibition experiments. Each value recorded represents the average of two or more determinations.

Dose	Output HCl (meq /3 hr)	Reduction (%)
Hog gastrin (50 mg)	1.55	
Hog gastrin (50 mg) + rabbit anti-hog gastrin serum (3 ml)	0.61	61
Hog gastrin (50 mg) + rabbit anti-hog gastrin serum (6 ml)	0.51	67
Hog gastrin (50 mg) + normal rabbit serum (3 ml)	1.29	17
Hog gastrin (50 mg) + rabbit anti-hog Group A substance serum (3 ml)	1.32	15
Dog gastrin (75 mg)	3.53	
Dog gastrin (75 mg) + rabbit anti-hog gastrin serum (3 ml)	0.90	75
Human gastrin (30 mg)	1.08	
Human gastrin (30 mg) + rabbit anti-hog gastrin serum (3 ml)	0.39	64
Human gastrin (30 mg)	1.92	
Human gastrin (30 mg) + normal rabbit serum (3 ml)	1.87	3
Histamine phosphate (1 mg)	1.93*	
Histamine phosphate (1 mg) + rabbit anti-hog gastrin serum (3 ml)	2.06*	

* Output in 90 minutes.

Secretion in response to the subcutaneous injection of gastrin normally starts within 30 minutes after the injection and has practically ceased after 3 hours. Accordingly, gastric juice was collected over a 3-hour period after the injection, and the total output of hydrochloric acid during this period was determined by titration with 0.1*N* sodium hydroxide with phenol red indicator.

Table 1 presents the data of several secretory inhibition experiments. Each value recorded represents the average of two or more determinations with the gastrin preparations indicated.

Incubation of 50 mg of hog gastrin with 3 ml of rabbit antiserum to hog gastrin caused a 61-percent reduction in the acid secretion produced by 50 mg of hog gastrin alone, and the reduction was slightly greater when the hog gastrin was incubated with 6 ml of serum. In contrast with this, incubation of gastrin with normal rabbit serum produced only a 17-percent reduction in secretion. That this reduction represents a nonspecific action of rabbit serum is suggested by the similar reduction achieved by incubation of hog gastrin with rabbit anti-hog Group A substance serum (Ortho Corp.). Gastrin prepared from dog and human stomachs was also reacted with rabbit anti-hog gastrin serum, and the secretory response was reduced by 75 percent and 64 percent, respectively.

To exclude the possibility that the anti-gastrin serum was producing inhibition of secretion by some mechanism other than the inactivation of gastrin, a portion of serum was incubated with a solution of histamine, and the mixture was injected subcutaneously. Rabbit anti-hog gastrin serum failed to depress the acid secretion induced by histamine.

The reaction of hog gastrin to rabbit anti-hog gastrin serum was studied in agar gel diffusion plates. One major antigenic component and at least three minor antigenic components were demonstrated, emphasizing the impurity of gastrin prepared by this method. Dog and human gastrin reacted with homologous rabbit antiserum similarly showed multiple antigenic components. An attempt was made to demonstrate a cross reaction between hog, dog, and human gastrins when they were reacted with rabbit anti-hog gastrin serum. No such cross reaction could be shown in agar gel diffusion plates in spite of the cross reaction achieved in the inhibition experiments described above. These results may be interpreted to mean that the active secretory principle in gastrin prepared by the Gregory method is in too low a concentration to form a cross-reacting precipitin band demonstrable by the relatively insensitive agar gel diffusion method (*7*). Another interpretation might be that not all antigen-antibody complexes precipitate, particularly if the antigen molecule is small or has only one antigenic site. Thus, gastrin may be a small, univalent, antigenic molecule.

The following conclusions are suggested. Gastrin produced by the Gregory method is a relatively impure substance with several demonstrable antigenic components. The active acid secretory principle contained in this preparation may be in low concentration or may be a small, univalent antigen which does not stimulate the production of precipitating antibody. Antiserum reacted with crude hog gastrin is capable of neutralizing the physiologic effect of hog, dog, and human gastrin, illustrating a cross reaction among the species mentioned. Gregory's method of preparing gastrin almost certainly excludes contamination of the final product with histamine which stimulates gastric secretion. However, the possibility that trace amounts of histamine might be present has been considered despite the fact that biologic assay does not reveal any histamine (*4*). The present experiments add further evidence that, in gastrin prepared by the Gregory method, histamine is not the substance that induces acid secretion (*8*).

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Erythrocyte Automosaicism in Some Persons of Known Genotype

Abstract. We have estimated the proportions of non-A and non-B erythrocytes in some members of four families in which both parents were blood group AB, including two B homozygotes. The exceptional cell frequencies were not discernibly correlated with age. The B homozygotes had fewer non-B exceptional cells than their AB parents or sibs, but too many to represent independent losses of the B alleles. These results are not in agreement with the simplest expectations based on the mutational hypothesis of automosaicism.

Red cell populations in human A or AB individuals and in *Phaseolus* lectin-positive pigeons are heterogeneous, containing a proportion of non-A or lectin-negative cells that is rather stable, but subject to individual variation. This proportion is increased by P^{32} treatment in man or by whole-body x- or gamma irradiation in pigeons (*1*). Part of the induced increase is stable for an indefinite time, which suggests that the phenotypic heterogeneity among the red cells reflects a corresponding genetic diversity among the reproducing erythropoietic cells. The presence of such exceptional cell populations generated within an individual has been called automosaicism to distinguish it from chimerism involving admixture of cells from different zygotes.

Two criteria bearing on the spontaneous origin of automosaicism are its relations to age and to zygosity. If it is mainly caused by accumulation of genetically altered stem cells during adult life, a correlation with age is expected. If it is caused by independent events affecting the two ABO alleles in the diploid cell, then the proportion of exceptional cells in a homozygote should approximate the square of the proportion in a comparable heterozygote.

Through the cooperation of the Chicago Blood Donors Association, we were able to study four families in which both parents in each family are group AB. These included two B homozygotes and related persons of widely disparate age. The genotypes and ages of the persons studied are shown in the figure captions. The experiments indicate, first, that non-A and non-B cell proportions in AB children are rather similar to those in adults; second, that the proportions of non-B cells in the B homozygotes, although from 1/5 to 1/20 of those in related heterozygotes, were still from 50 to 200 times higher than the squares of the proportions in the heterozygotes.

The proportions of non-A and non-B cells were estimated by the isotope dilution method previously described (*2*). Saline suspensions of the cells were labeled with Cr^{51} and passed through repeated agglutinations and separations (stages) in the presence of unlabeled "carrier" cells. Within five or six stages the rate of removal of labeled cells per stage usually becomes negligible and the remaining proportion of labeled cells approximates the true inagglutinable fraction. In the present experiments, unlike those previously reported, human anti-A and anti-B sera were used, rather than lima bean anti-A lectin. The sera were obtained from donors selected for the production of commercial typing sera, unselected sera having proved unsatisfactory. In all experiments with anti-B, B cell carrier was used, whereas A_1 carrier was used in all those with anti-A.

Isotope dilution curves with anti-B were obtained for the AB and B persons in three of the families (Fig. 1). The AB persons show no consistent age effect. The results in family II suggest the possibility of a non-B frequency characteristic of the B allele, with Br.O. having received his mother's B allele and Wa.O. his father's. This interpretation does not find further support in family III, nor in the non-A proportion in family II; we regard the result as fortuitous. The B homozygotes are distinct; De.O. has a non-B frequency 10 to 20 times lower than in sibs and parents, and Ka.F. has 5 to 10 times lower.

The results with anti-A are shown in Fig. 2. Evidently the proportions of non-A and non-B cells can be quite