Concentration and Partial Characterization of Human Salivary Gastrone

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A gastric secretory depressant called gastrone,1 present in human gastric juice and active on intravenous injection, was demonstrated first by Brunschwig et al. in 19392; similar inhibitors have since been found also in canine gastric juice,3 in porcine gastric juice,4 in human saliva,5 in canine saliva,6 and in fasting canine thoracic duct lymph.7 Partial fractionation of pooled human gastric juice8 has demonstrated that there are two active inhibitors in that secretion, one a gamma globulin with antibody activity against parietal cells, the other a compound which on certain electrophoresis exhibits mobility and staining properties suggestive of a sialomucin.

Human saliva is the most readily obtainable of all the gastrone-containing secretions, but to date it has not been fractionated in an attempt to isolate or identify the active substance or substances. This report describes a series of experiments which has enabled us to concentrate salivary gastrone activity and partially to characterize the active factor in a pool of saliva from normal subjects. These results provide a basis for further separation and analysis of salivary gastrones.

METHODS

The pool of saliva was obtained from laboratory-staff members, male children in an orthopedic ward, and adult paraplegics in a rehabilitation unit; all subjects were free from gastrointestinal disease. Paraffin-stimulated mixed saliva was pooled, dialyzed for 48 hr. against distilled water at 4°, lyophilized, homogenized, and stored at room temperature.

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Each gram of lyophilized saliva was extracted with 100 ml. phenol (90% w/v) substantially as described by Morgan and his co-workers. After 3 extractions, each of 30 min., phenol was removed from the pooled supernatants and from the final phenol-insoluble residue by dialysis against multiple changes of distilled water for a total of 120 hr. at 4°. Each of the fractions obtained was then lyophilized, homogenized, and stored at room temperature.

Biological assay of the products of phenol fractionation demonstrated that all gastrone activity remained in the phenol-insoluble material (vide infra), to which subsequent preparative procedures were confined. This was digested for 24 hr. at 37° with an excess of nonspecific protease* after which the digestion mixture was boiled for 15 min. to destroy residual enzyme activity and then centrifuged at 1750 x g for 10 min. The supernatant was dialyzed for 48 hr. against distilled water at 4° to remove small-molecular-weight products of proteolytic digestion and was then lyophilized.

Biological assay of gastrone activity was performed at each stage, using the pylorus-ligated rat preparation described initially by Shay et al. and used since that time for assay of gastrone activity. Adult Sprague-Dawley rats of 100-250 gm. received an injection of control or test substance into a lateral tail vein at the time of pylorus ligation, after which a collection period of 4 hr. elapsed before the stomach was excised and the animal sacrificed. Any sample of gastric juice containing blood or feces was excluded from the study. The acid contents of the remaining samples were measured as previously described and used for the comparison of groups. All statistical comparisons were made using a rank-sum test.

The detailed protocols of the assays of gastrone activity in saliva and its fractions are shown in Table 1. Lyophilized material to be tested was added to solvent in the concentrations shown in Table 1. After mixing for 10 min., undissolved material was removed by centrifugation at 1750 x g for 10 min. and only the supernatant was injected.

The pylorus-ligated rat was also used to construct dose-response curves of the effect of whole saliva and of phenol-insoluble, protease-digested, and dialyzed (PIPD) extract on rat gastric acid secretion. Lyophilized whole saliva was added to 0.1 M phosphate buffer pH 7.4 in amounts ranging from 0 to 6.4 mg./ml. Undissolved material was removed by centrifugation and the supernatants were injected into groups of 4-6 male rats (weight 90 to 180 gm.) in a volume of 20 ml./kg. in each case. Doses thus ranged from 0 to 128 mg. saliva per kilogram of rat weight. Lyophilized PIPD saliva extract was reconstituted in the same way and groups of rats of the same size and weight were injected with equal volumes containing doses of 0-16 mg. lyophilized material per kilogram of rat weight. Gastric juice was collected at the end of 4 hr. and the

* Nonspecific purified fungal protease from *St. griseus*, Sigma Chemical Company, St. Louis, Mo., U.S.A.