Histochemical Study of the Mucosubstances in the Canine Stomach

I. The Resting Mucosa

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A LTHOUGH gastric secretions in the dog have been the subject of numerous chemical analyses,¹⁻⁷ corresponding morphologic and histochemical data on the gastric mucosa are sparse.

Chemical studies of the gastric juice indicate the presence of a neutral glycoprotein⁵ which represents the major component here as in other epithelial secretions,⁶ sialic acid,¹ ⁴ ⁷ and a sulfated component which had been once designated "mucoitin sulfuric acid. "³ ⁸ Recent investigations of gastric mucosal extracts from the dog⁹ have demonstrated two sulfate-containing fractions, one of which is a sulfated glycoprotein, said to be of epithelial origin, and the other, chondroitin sulfate B, allegedly derived from connective tissue. Serum protein¹⁰ has been identified in dog gastric secretion, but the protein moiety of the mucin molecule itself has never been characterized chemically.

In 1941 Zanotti¹¹ proposed a histochemical classification of canine gastric mucins based on the chromic acid-Schiff reaction for neutral glycoproteins and on the use of metachromatic dyes to demonstrate sulfate groups. A more detailed study based on newer histochemical¹² and autoradiographic¹³ technics has been performed recently by the senior author.¹⁴ He found that the fundic and antral crypt cells contain both sulfate and carboxyl groups, whereas the more superficial mucous cells contain a high concentration of a carboxylated substance, possibly sialic acid.

The current study will be concerned with a better histochemical differentiation of the acidic and neutral carbohydrate and the protein moieties of the epithelial mucins of the gastric mucosa in the dog and will attempt to demonstrate the cellular origin of some of the fractions isolated chemically from gastric secretions.

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MATERIALS AND METHODS

Fundic and antral mucosal biopsies were obtained from 17 mongrel dogs weighing from 5 to 15 kg., following a 24 to 48-hr. fasting period. The specimens were obtained from anesthetized dogs through a gastrotomy incision or from unanesthetized animals bearing permanent gastric fistulae; a standard biopsy forceps through a sigmoidoscope was used. Normal human gastric fundic mucosa was obtained from 4 freshly fixed gastrectomy specimens; the surgery was performed because of duodenal ulcer. The specimens were fixed for 6 hr. in neutral buffered formalin, Bouin's solution, Carnoy's fluid, or Regaud's solution, then embedded in paraffin and sectioned at 5 μ.

Neutral glycoproteins were identified by the periodic acid-Schiff reaction, and acidic glycoproteins by the following basic dyes: (1) 0.1% toluidine blue (TB) in a 0.1 M citrate buffer at pH 3.0; (2) 1% alcian blue (AB) at pH 2.5; (3) colloidal iron (CI), according to Mowry's modification of the Hale technic; and (4) aldehyde fuchsin (AF), according to Gomori. Sulfated mucins were selectively demonstrated by: (1) 1% AB in 2 N H₂SO₄ at pH 0.5; (2) TB in 0.1 N HCl at pH 1.0; (3) the high iron diamine (HID) stain; and (4) in-vivo autoradiography with Na₂³⁵SO₄.* In the latter procedure, tissue sections were prepared from biopsies taken 1, 4, 24, 48, and 96 hr. following intravenous injection of 1 mc./kg. of the isotope. Some of these sections were then stained with AB (at pH 2.5 or 0.5) or the PAS reaction, preceded in certain cases by hyaluronidase digestion or methylation at 60°, the technics for which are explained below. The stripping-film technic was then applied to these sections, which were subsequently exposed for 2 weeks at 4° prior to developing.

In certain cases AB (pH 0.5 or 2.5) was combined with the PAS reaction in order to stain the acidic and neutral moieties of the glycoprotein molecule blue and red respectively in the same section. In some instances, a HID-AB (pH 2.5) sequence was used in order to depict sulfomucins (black) and sialomucins (blue) simultaneously. The relationship of the vicinal hydroxyl and acidic moieties of the mucins was explored with the periodic acid-phenyl-hydrazine-Schiff (PAPS) and periodic acid-N, N-dimethyl-phenylenediamine (HCl)₂-AB at pH 2.5 (PA meta D-AB) procedures. Periodic acid oxidation followed by N, N-dimethyl p-phenylenediamine HCl treatment (PA para D) was also used.

Protein was demonstrated with Biebrich scarlet at pH 5.6 and 9.5, bromphenol blue, the ninhydrin-Schiff reaction, and the coupled tetrazonium reaction. Sulfur-containing protein was identified using the dihydroxydiphenyl-disulfide procedure (DDD) of Barrnett and Seligman with and without prior thioglycollate reduction and by the HID reaction following a 15-min. oxidation with a 1% solution of acidified potassium permanganate.*

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