Secretion of a Chondroitin Sulfate-Like Substance by the Chief Cells of the Dog Gastric Mucosa.* (31926)

ANDRÉ GERALD,† JACQUES DEGRAFF,† ROBERT LEV AND GEORGE B. JERZY GLASS

Gastroenterology Research Laboratory and Section of Gastroenterology, Departments of Medicine and of Pathology, New York Medical College, New York City

A sulfated compound, previously designated "mucoitin sulfuric acid" by Levene and Lopez-Suarez(1) was found by Komarov(2) in the dog gastric juice more than 30 years ago, and by Meyer *et al*(3) and Werner(4) in the hog gastric mucosa. Others found that "mucoitin sulfuric acid" from hog gastric mucosa represented a mixture of chondroitin sulfates and heparin(5). In 1964, one of us (J. DeG.)(6) showed by paper electrophoresis a compound moving and staining like acid aminopolysaccharide (AAP) in the gastric juice collected from a fundic pouch in the dog. In their 1965 study of the canine stomach, Hakkinen *et al*(7) reported on 2 groups of sulfated compounds, of which one contained chondroitin sulfate (CHS) B presumably of connective tissue origin, and the other, sulfated glycoprotein, presumably derived from gastric epithelium.

This study was designed to determine the nature and source of origin of the sulfated AAP in the canine stomach.

Material and methods. Gastric juices were collected from Heidenhain and Pavlov pouches from 5 mongrel dogs (6-15 kg wt) after i.v. urecholine stimulation (1-2 mg/hr/10 kg). AAP was separated as follows: Dialyzed and lyophilized gastric juices were digested with papain, precipitated with cetyl pyridinium chloride, dissolved in 1 M $MgCl_2$, reprecipitated with 4 volumes 95% alcohol, washed with alcohol, dissolved in water and lyophilized. Lyophilizates were subjected to chemical analysis and electrophoresis on cellulose acetate strips in a borate buffer, pH 9.0 $\Gamma/2$ 0.24. Further details of this technic will be published separately(8).

Chemical analysis of this material was performed as follows: Hexosamine was determined by the method of Rimington(9), uronic acid by that of Bitter and Muir(10) and sulfates by the method of Dodgeson and Spencer(11). The effect of the digestion with hyaluronidase on this material was studied and quantitated by the method of DiFerrante (12).

Histochemical investigation of gastric fundic mucosa obtained by biopsy through gastric fistulae in 17 mongrel dogs (6-15 kg wt) was performed on specimens fixed with buffered formalin. The histochemical reactions applied to this tissue included: (a) Basic dyes: 0.1% toluidine blue at pH 1.0 in 0.1 N HCl or at pH 3.0 in 0.1 M citrate buffer; alcian blue at pH 0.5 or 2.5(13); aldehyde fuchsin(14); and the high iron diamine stain for sulfomucins(15); (b) Periodic acid oxidation followed either by N,N-dimethyl-p-phenylenediamine HCl(15), or by N,N-dimethyl-m-phenylenediamine $(HCl)_2$ and alcian blue (pH 2.5); (c) Periodic acid-Schiff PAS reaction(16); (d) Methylation using absolute methanol containing 0.1 N HCl for 3 hours at 60°C, and (e) Digestion with 0.05% testicular hyaluronidase (Worthington) at pH 5.5 for 4 hours at 37°C.

The uptake of radioactive $Na^{35}SO_4$ by the gastric mucosa was determined *in vivo* by radioautography of the mucosa of the stomach biopsied 1-4 hours after i.v. injection of 1 mc/kg body wt of $Na^{35}SO_4$.

Results and discussion. Chemical analysis of the AAP precipitated from the dog gastric juice revealed the presence of hexosamine, uronic acid and sulfates in a mean molar ratio of 100.0:95.5:73.3 (Table I). When this material was incubated with testicular hyaluronidase and then tested for AAP, 85-95% (mean 92.3%) of it became completely digested, as is the case with CHS A or C(17).

*This work was supported by Research Grants-in-Aid AM-09701 and HD-01666-01 from Nat. Inst. of Arthritis & Metab. Dis., NIH, USPHS.

†Present address: Service de Chirurgie Générale, Hôpital Universitaire Saint Pierre, Brussels, Belgium.

TABLE I

	Dog #	Molar ratio of uronic acid and sulfates to hexosamine = 100.0		
		Hexosamine	Uronic acid	Sulfates
Sulfated AAP separated from dog gastric juices	703*	100.0	108.0	81.5
	"	"	105.0	82.0
	708†	"	96.0	76.0
	1034*	"	97.0	75.0
	705*	"	80.0	60.5
	709*	"	94.0	65.0
	Mean	100.0	95.8	73.3
Chondroitin sulfate sample		100.0	101.0	72.5
Heparin sample		100.0	100.0	227.0

* Heidenhain pouch. † Pavlov pouch.

When the same material was electrophoresed, it consisted of one major component, moving towards the anode with the electrophoretic mobility similar to that of CHS, and a more slowly moving minor component (Fig. 1). The major component stained with alcian blue at pH 1.5 (Fig. 1) and with aldehyde fuchsin, showed slight metachromasia with toluidine blue and azure A at pH 1.5 and did not stain with the PAS stain. It thus possesses the staining properties of a sulfated AAP and the electrophoretic mobility of a CHS-like material. These findings suggest that the major sulfated AAP in urecholine stimulated dog fundic juice is very similar in its chemical and electrophoretic characteristics to CHS A or C.

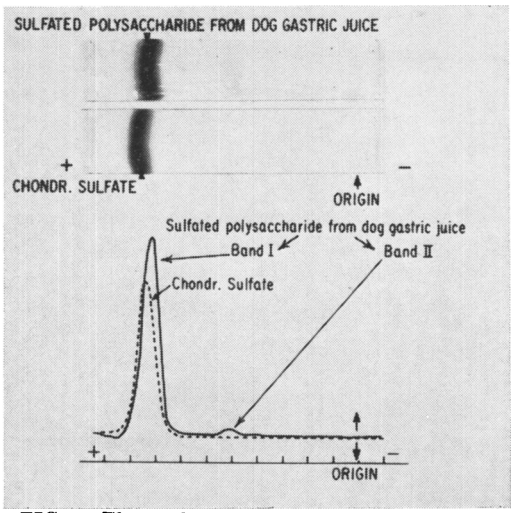

FIG. 1. Electrophoretic pattern on cellulose acetate of chondroitin sulfate and sulfated acid aminopolysaccharide separated from fundic pouch gastric juice of a dog. (Alcian blue at pH 1.5.)

Histochemical investigation of biopsied fundic gastric mucosa revealed a similar material in the cytoplasm of the chief peptic cells. These exhibited the following characteristics: (a) alcianophilia and metachromasia at pH 0.5-1.0, (b) alcianophilia at pH 2.5 which persisted after treatment with periodic acid, N,N-dimethyl-m-phenylenediamine, (c) affinity for aldehyde fuchsin, (d) positive reaction with the high iron diamine procedure which is specific for sulfated mucosubstances (15), (Fig. 2A), (e) uptake of radioactive sulfate (Fig. 3A), (f) no staining with PAS even after methylation with methanol-HCl, (g) slow appearance of a black color with periodic acid N,N-dimethyl-p-phenylenediamine (h) elimination of the bulk of high iron diamine and basic dye staining (Fig. 2B) and $^{35}SO_4$ uptake (Fig. 3B) by testicular hyaluronidase digestion.

These histochemical findings suggest the presence of a sulfated AAP in chief peptic cells. This substance is presumably CHS A or C since it is susceptible to testicular hyaluronidase digestion. Such a material has not previously been identified unequivocally in an epithelial cell.

This conclusion is corroborated by other findings in our laboratory(8) obtained in dogs with fundic Heidenhain or Pavlov pouches. Following stimulation of gastric secretion with intravenous infusion of urecholine (1-2 mg/hr/10 kg) superimposed on sub-maximal i.v. histamine drip, a histochemically demonstrable depletion of the CHS-like material and pepsinogen granules from peptic cells was observed. This was associated with a parallel

increase in the concentration and output of a CHS-like substance and pepsin in the gastric juice(8). In view of the known inhibitory effects of sulfated polysaccharides on peptic digestion, the presence of a sulfated AAP, potential pepsin inhibitor, in the pepsinogen producing cells may be significant. It may possibly prevent the reaction between the activated zymogen and the substrate (gastric mucosa) before pepsin is discharged into the gastric lumen.

We did not find similar sulfated AAP in any other epithelial cells of the dog gastric fundic mucosa. The AAP material detected in chief cells differs histochemically from the sulfomucin present in the fundic crypt epi-

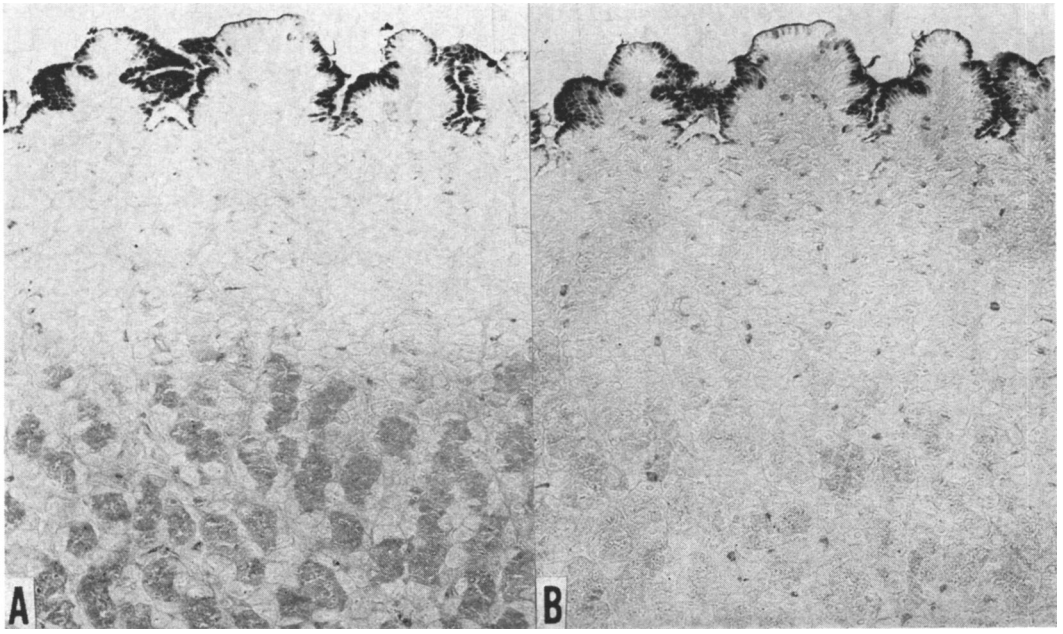

FIG. 2. Dog fundic mucosa stained with high iron diamine stain for 16 hr; 150 ×. a) Untreated. Note grey staining of the chief cells in lower half of photograph and intense black staining of crypt and surface epithelium above. b) After 4 hr digestion with testicular hyaluronidase prior to staining. Chief cell staining is virtually eliminated whereas crypt and surface epithelium exhibit the same degree of dye uptake.

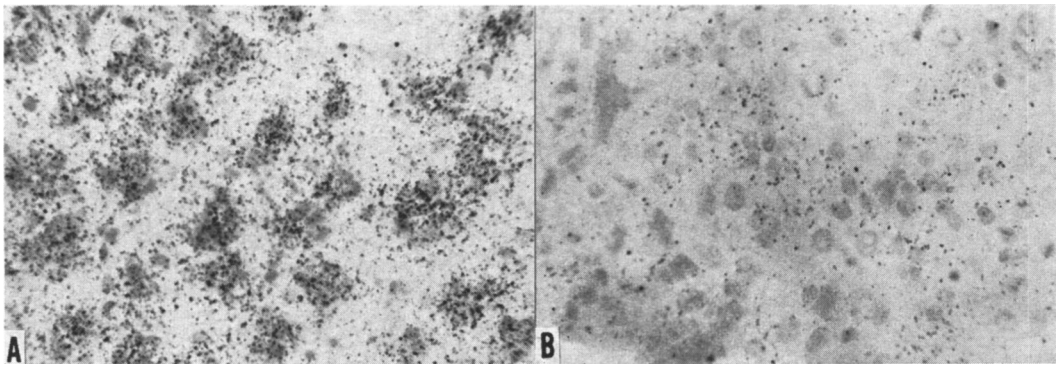

FIG. 3. Autoradiography of the dog fundic mucosa biopsied 4 hr after intravenous injection of 1 mc/kg $Na_2{}^{35}SO_4$, stained with alcian blue at pH 2.5, then subjected to the stripping film technique, exposed for 2 weeks, and developed. a) Untreated. Note high concentration of silver granules around clusters of chief cells. b) Treated for 4 hrs with hyaluronidase prior to alcian blue staining, otherwise processed as in 3A. Note marked reduction in number of silver granules around clusters of chief cells.

thelium. The latter compound is PAS positive, its sulfate staining is unaffected by testicular hyaluronidase, and it loses its alcianophilia after the periodic acid, N,N-dimethyl-m-phenylenediamine treatment. These histochemical properties mitigate against the presence of a CHS-like material in the fundic crypt epithelium, suggesting the existence of a sulfated glycoprotein instead. Such a material may be akin to sulfated glycoproteins chemically separated from dog gastric mucosa (7).

Summary. The correlation of chemical, electrophoretic and histochemical findings herein reported is consistent with the concept that the chondroitin sulfate A- or C-like material found in canine fundic gastric juice is derived from chief peptic cells.

1. Levene, P. A., Lopez-Suarez, J., J. Biol. Chem., 1916, v25, 511.
2. Komarov, S. A., ibid., 1935, v109, 177.
3. Meyer, K., Smyth, E. M., Palmer, J. W., ibid., 1937, v119 73.
4. Werner, J., Acta Soc. Med. Uppsala., 1953, v17, 1
5. Smith, H., Gallop, R. C., Biochem. J., 1953, v53, 666.
6. DeGraef, J., La sécretion de protéines et de glycoprotéines par la muqueuse gastrique fundique chez le chien, Ed., Arscia, S. A., Bruxelles, 1964, 222 pp.
7. Hakkinen, I., Hartiala, K., Terho, T., Acta Chem. Scand., 1965, v19, 800.
8. DeGraef, J., Glass, G. B. J., In preparation.
9. Rimington, C., Biochem. J., 1940, v34, 931.
10. Bitter, T., Muir, H. M., Analyt. Biochem., 1962, v4, 330.
11. Dodgeson, K. S., Spencer, B., Biochem. J., 1953, v55, 436.
12. DiFerrante, N., J. Biol. Chem., 1956, v220, 303.
13. Mowry, R. W., J. Histochem. & Cytochem., 1956, v4, 407.
14. Gomori, G., Am. J. Clin. Path., 1959, v20, 665.
15. Spicer, S. S., J. Histochem. & Cytochem., 1965, v13, 211.
16. Lille, R. D., McGraw-Hill, New York, 3rd ed., 1965.
17. Meyer, K., Rapport, M. M., Science, 1951, v113, 596.

Received January 3, 1967. P.S.E.B.M., 1967, v124.

Effects of Thyroid Status and Adrenergic Blocking Drugs on Isoproterenol-Induced Enlargement of the Salivary Glands.* (31927)

GEORGE A. BRAY (Introduced by E. B. Astwood)
New England Medical Center Hospitals and The Department of Medicine, Tufts University School of Medicine, Boston, Mass.

There is a substantial body of evidence suggesting that the response of certain adrenergic receptor mechanisms is altered by thyroid status whereas other responses are unaffected(1-3). For example catecholamine-induced lipolysis *in vitro* is diminished in adipose tissues from hypothyroid rats(4) and enhanced in adipose tissue from hyperthyroid rats(5). Similarly depletion of cardiac glycogen by isoproterenol is diminished in hypothyroid rats(3). Studies from several laboratories have shown that isoproterenol increases the wet and dry weight of submaxillary and parotid glands of the rat(6-8) and that this response is inhibited by dichloroisoproterenol(9). The following experiments were conducted to investigate the effect of thyroid status and adrenergic blocking drugs on the response of rat salivary glands to isoproterenol.

Methods and materials. The male Holtzman rats used in these experiments were housed in a constant temperature room and fed Purine Labena Chow and tap water *ad lib.* Thyroidectomy was performed as previously described(10) at least 4 weeks before the beginning of an experiment and the adequacy of operation was evaluated by cessation of growth(11). A diet containing 2.5% pancreatin (Viokase) was fed for 6 days. Isoproterenol was diluted each day from com-

*This work was supported by Grant AM-5166-08 from USPHS.