TABLE I.
More Recent Experiments on Effect of Hyaluronidase on Adhesion Formation Due to Mechanical Trauma.

Agent (applied locally)	Total	% adhesions in treated rats	% adhesions in control	Instrument used to produce adhesions
5% hyaluronidase (Schering)	19	42.1	76	Allis
5% hyaluronidase (Schering)	9	88.9	100	Payr
1250 TRU hydase (Wyeth)	17	94	100	Kelly
1250 VRU alidase (Searle)	10	100	100	"

TRU = Turbidity reducing units.
VRU = Viscosity reducing units.

While it is not possible with the data now available to prove that hyaluronidase will not exert an effect under some other experimental conditions, our inability to extend the original observations in this series of experiments has led us to believe that hyaluronidase, in the rather liberal doses used, has no general applicability in the prevention of intraperitoneal adhesions in the rat.

Conclusions. Adhesions were produced in the rat by mechanical trauma to the wall of the cecum by 3 standardized methods. The effect of 3 hyaluronidase preparations applied locally to the injured area at the time of laparotomy was tested. One preparation was tested against 2 types of injury and the other 2 preparations were tested against a third type of injury. No statistically significant effects were obtained to support the positive results previously reported by 2 of us.

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Polysaccharides in Ulcerative Colitis and Normal Human Rectum Compared by Stains of Biopsy Specimens. (17952)

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The hypothesis of Meyer, Gellhorn, Prudden, Lehman and Steinberg(1) that the enzyme lysozyme(2) plays a role in the pathogenesis of chronic ulcerative colitis depends in part on the demonstration of a lysozyme substrate in the colon. These workers found extremely high titers of lysozyme in the feces of patients with chronic ulcerative colitis. We have also observed an elevated output of lysozyme in the feces of all patients with ulcerative colitis tested so far. As yet, Meyer (1), Jerzy Glass(3), and others(4) have been unable to demonstrate a lysozyme substrate in the bowel or in the surface mucus from the colon. Wang and coworkers(4) report damage of the mucosa and submucosa by lysozyme placed in the lumen of the bowel of rats. This report concerns the demonstration of a lysozyme substrate by the histochemical method of McManus(5) or Hotch-

1. Meyer, K., Gellhorn, A., Prudden, J. F., Lehman, W. L., and Steinberg, A., (a) PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 221; (b) *Am. J. Med.*, 1948, v5, 496.

2. Fleming, A., and Allison, V. D., *Brit. J. Exp. Path.*, 1922, v3, 252.

3. Jerzy Glass, G. B., Pugh, B. L., Grace, W. J., and Wolf, S., *J. Clin. Invest.*, 1950, v29, 12.

4. Wang, K. J., Grant, R., Janowitz, H. D., and Grossman, M. I., *Arch. Path.*, 1950, v49, 298.

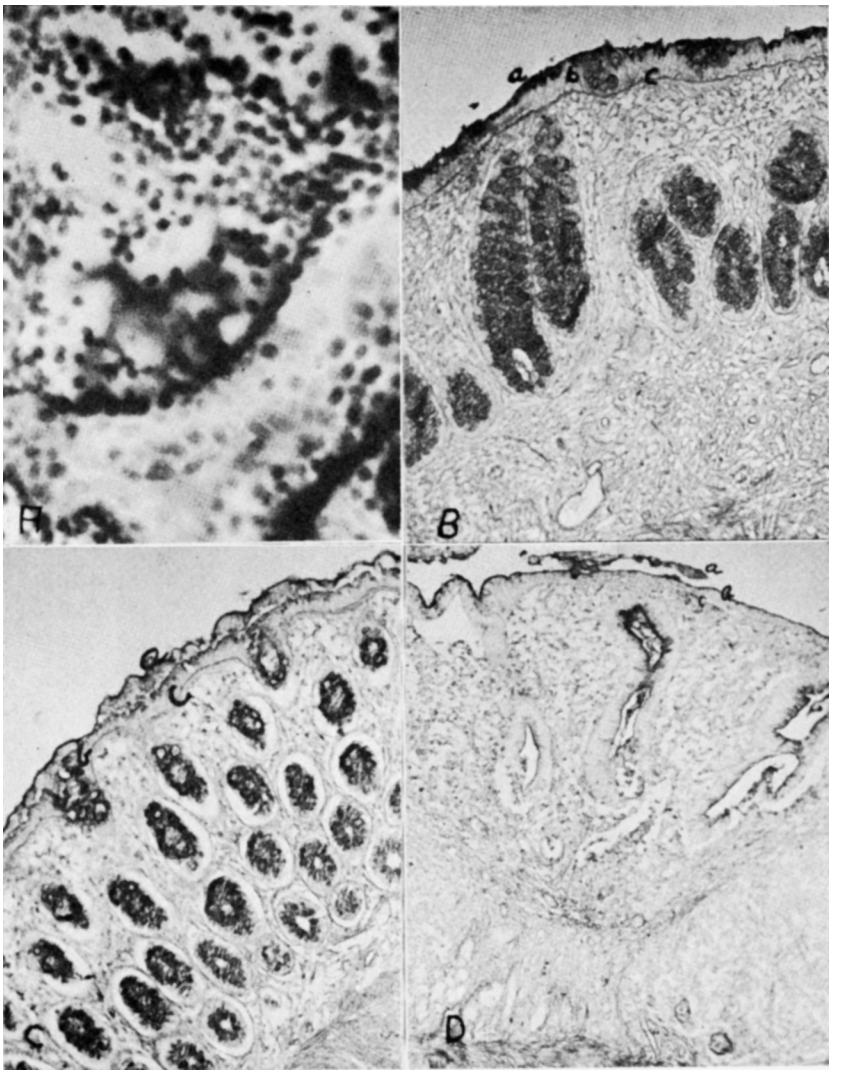

FIG. 1A, B, C, D. (All McManus stain only).
(A) *Micrococcus lysodeikticus* fixed in gelfoam, infiltrated with paraffin, sectioned and stained by the McManus method. (1367×). (B) Normal rectum stained by the McManus method. (80×) (a—surface polysaccharides; b—intracellular accumulations; c—basement membrane). (C) Section of rectum from a patient with chronic ulcerative colitis. (80×) (a—surface polysaccharides; b—intracellular accumulations; c—basement membrane). (D) Section of rectum from a patient with chronic ulcerative colitis. (80×) (a—surface polysaccharides; b—intracellular accumulations; c—basement membrane).

kiss(6) and investigations to determine whether or not this substrate is present in human rectum.

Method. Surface growth of the organism, *Micrococcus lysodeikticus,* was embedded in gelfoam and fixed in 10% formalin, 100% alcohol and acetone. The 3 specimens were infiltrated with paraffin and 6-12 μ sections were cut. Sets of sections from each fixative were attached to slides with gelatin and egg

5. McManus, J. F. A., *Nature,* 1946, v158, 202.
6. Hotchkiss, R. D., *Arch. Biochem.,* 1948, v16, 131.

albumin respectively. The slides were kept at 60°C for 1 hour, the paraffin removed and stains applied. Biopsies from areas of bowel with active disease were taken at proctoscopy in 10 patients with ulcerative colitis. Biopsies were similarly taken from the bowel of patients without organic disease. Some of these specimens were put in lysozyme (0.5 mg crystalline egg white lysozyme / cc saline for 12 hours) before and after fixation. All specimens were fixed in 10% aqueous formalin, 100% alcohol or acetone. After fixation, sections were made and stained with the McManus or Hotchkiss technic. Hematoxylin and eosin preparations also were made. Fixed tissues were also incubated in hyaluronidase (50 TRU/cc buffer pH 6.5) for 20 hours. Amylase was used to prevent the staining of glycogen.

Results. (1) Surface growth of *Micrococcus lysodeikticus* as prepared by this method revealed the organisms to be brilliantly stained with the McManus or Hotchkiss method for polysaccharides. This was true for all methods of fixation and whether the sections were attached to the slide with gelatin or with egg albumin (Fig. 1A).

(2) Treatment of deparaffinated sections of these prepared organisms with lysozyme revealed a complete loss of the ability of the organisms to take on this polysaccharide stain, whereas the saline control sections showed no loss of the polysaccharides. This was found to be true for specimens prepared in all fixatives.

(3) Polysaccharide substances are present in the normal rectum as: (a) a well-defined surface layer, (b) a basement membrane, (c) dense intracellular accumulations in the mucosa, (d) part of the connective tissue fibers, muscle bundles and vascular walls (Fig. 1B).

(4) The surface layer and intracellular accumulations in the mucosa in sections of ulcerative colitis do not appear to differ from the normal (Fig. 1C). The basement membrane in the 10 specimens of ulcerative colitis appeared undisturbed with one exception, in which it seemed much fainter and thinner than normal (Fig. 1D).

(5) Lysozyme applied to normal and ulcerative colitis specimens before fixation and after fixation and sectioning, produces no demonstrable change in the polysaccharides as measured with the McManus-Hotchkiss stain. A biopsy taken from the same individual as was the specimen in Fig. 2 was incubated in lysozyme before fixation and after fixation and sectioning. It appeared identical to the specimen in Fig. 2.

(6) Incubation of bowel sections in hyaluronidase produced no demonstrable changes in stained tissue.

Comments. A specific substrate of lysozyme can be easily demonstrated with the McManus-Hotchkiss stain for polysaccharides. It has been shown with this histochemical method that the substrate disappears after treatment with lysozyme. Many polysaccharide substances are demonstrable in the bowel but none of these are destroyed by lysozyme used in the same manner as that employed to destroy the specific substrate found in the *Micrococcus lysodeikticus*. These observations suggest that the human rectum contains no specific substrate for the enzyme lysozyme.

Summary. 1. The lysozyme substrate contained in *Micrococcus lysodeikticus* is stained by the Hotchkiss or McManus technic after fixation and sectioning by the same procedure utilized for tissue. After fixation and sectioning, the substrate remains susceptible to lysis by lysozyme. Egg albumin, as used in application of sections to slides, does not lyse the lysozyme substrate.

2. Biopsy specimens of rectal mucosa from normal persons and patients with active ulcerative colitis after various methods of fixation show no consistent difference in reaction to the Hotchkiss or McManus stain.

3. *In vitro* application of lysozyme and hyaluronidase to human rectal tissue of normal individuals and patients with ulcerative colitis does not alter the reaction of the tissue to the Hotchkiss or McManus stain.

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