Additional proof of the safety of such pools was provided by absence of live poliovirus from large samples withdrawn as much as 5 days prior to end of inactivation period, and also by use of these vaccines in the field.

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Inhibition of Pepsin and Lysozyme by Acidic Polymers (24599)

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A series of acidic macromolecules arising from polymerization of phenols, or phenolic carboxylic or sulfonic acids have been shown to inhibit ribonuclease and other hydrolytic enzymes(1). Certain members of the series likewise showed activity in suppressing formation of gastric ulcers in Shay rats.* We were interested to learn if this biological activity could be correlated with inhibitory power against pepsin and/or lysozyme. Levey and Sheinfeld(2) and Marini and Levey(3) reported that chondroitin sulfuric acid inhibits pepsin *in vitro*, and that this polymeric acid also reduces the number of ulcers formed in the Shay rat stomach. Whereas pepsin has long been implicated in the etiology of gastric ulcers(4) the participation of lysozyme was suggested by K. Meyer *et al.*(5) and has been doubted by Reifenstein *et al.*(6), who feel that the increased tissue lysozyme found in the margins of active, but not of chronic, gastric ulcers represents a tissue response to the lesions rather than an etiological factor. Reports on inhibition of lysozyme action by acidic polymers and related compounds have appeared from several laboratories; the substances include hyaluronic acid(7), treburon and heparin(8,9) alkyl sulfates, fatty acids and alcohols(5,10,11). *Polymers.* The 4 polymers discussed in this paper were prepared as described(1) by condensation of I. Formaldehyde and hydroquinone sulfonic acid. II. Formaldehyde and 3,4-dihydroxybenzene sulfonic acid. III. Furfural and hydroquinone sulfonic acid. IV. Acetaldehyde and hydroquinone sulfonic acid.

Methods. Measurement of lysozyme inhibition. The criterion most widely used to evaluate lysozyme activity, *viz.*, clearing of a suspension of *M. lysodeicticus*(12,13,14,15) probably is not reliable(16) when the reaction is allowed to proceed at pH2 followed by alkalinization(17). Optical methods appeared unattractive for study of inhibition by colored substances. Enzyme activity was measured by viscosimetry according to Meyer and Hahnel(18). The substrate (80 mg) was dissolved in 30 ml of McIlvaine's buffer (pH 2.15) containing 0.2 M sodium chloride. A small amount of gummy residue was removed by centrifugation after the solution had been agitated for one hour. The solution may be kept for at least 8 hours at room temperature without change in viscosity. Twice recrystallized lysozyme (Worthington Biochemical Corp.) was dissolved in 0.9% sodium chloride to give 500 μg/ml. The polyphenolsulfonic acids used as inhibitors were dissolved in distilled water. Viscosimetry was patterned after the procedure used by Northrop for measurement of peptic activity(19). Four-ml aliquots of substrate solution were placed into 50-ml test tubes. To each tube, 0.5 ml

* Dr. A. Plummer of this Department observed the anti-ulcer effect in Shay rats. We thank Dr. Plummer for permission to refer to his results.

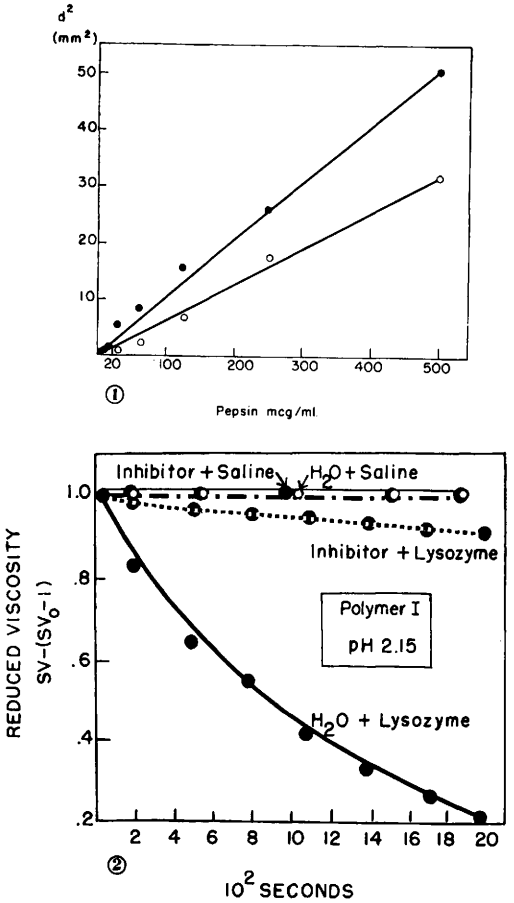

FIG. 1. Plot of square of distance of albumin digested (d^2) in Mett tubes during 16 hr at 37° by pepsin in 0.056 *N* HCl in presence (○) and absence (●) of 200 μg/ml of polymer I.

FIG. 2. Inhibition of lysozyme (50 μg/ml) action by 240 μg/ml of polymer I at pH 2.15.

of water or inhibitor solution was added and the mixture was well stirred. For enzymatic digestion a tube was equilibrated in a water bath at 35.5° ± 0.04. At zero time 0.5 ml of enzyme solution was rapidly mixed with the substrate solution, and the digestion mixture was introduced into an Ostwald viscosimeter submerged in the water bath. Total elapsed time as well as outflow time were recorded for each viscosity measurement. For measurements at pH 5.25, the optimum for lysozyme activity(18), the same procedure was used with suitably reduced concentrations (Fig. 3 and Table I) of enzyme and inhibitors. However, at pH 5.28 and 35.5° the substrate undergoes a slow non-enzymatic decrease in viscosity, correction for which was made accordingly. *Measurement of pepsin inhibition.* Formol titration of gelatine-pepsin digests, viscosimetry of edestine-pepsin solutions, and determination of non-protein nitrogen were not suitable to measure pepsin activity in the presence of the polymers under study. Only the solubilization by pepsin of coagulated egg white allowed us to compare, in an approximate fashion, the inhibition exerted by 3 of the polymers. Mett tubes were prepared from the liquid portion of egg white by coagulation in pyrex melting point capillaries as in the standard procedure(20). Twice crystallized pepsin (Worthington Biochemical Corp.) was used. Sections of the albumin tubes were dropped into solutions of enzyme with and without inhibitor and incubated at 37°. Degree of digestion was estimated by measuring, on a vernier microscope stage, the length of albumin column dissolved at each end of the tube sections. Fig. 1 shows that under the conditions chosen the proteolytic power of serial dilutions of pepsin in presence or absence of a fixed amount of inhibitor is proportional to the square of the length of the albumin column digested.

Results. (A) Viscosimetry. If t and t_{H_2O} are the outflow times in seconds, of solution and solvent, respectively, and SV is the specific viscosity, then

$$SV = \frac{t}{t_{H_2O}} - 1. \qquad (1)$$

Initial specific viscosity SV_0 was determined by extrapolation to zero time from time-viscosity graphs.

To facilitate comparison of the plots of different runs, the quantity (SV_0-1) was subtracted from each of the SV-values found in a run, so that all curves begin at 1.00 on the viscosity axis. The viscosity values so obtained were plotted against time, assigning to each point a time T equal to elapsed time plus one-half the outflow time measured. T = elapsed time + ½ t. Results are represented in Figs. 2 and 3. Initial rates were calculated approximately as per cent viscosity change per unit time occurring during the first 500 seconds.

TABLE I. Inhibition of Lysozyme (50 μg/ml) at pH 2.15.

Reaction mixture	t_o	t_{500}	Rate (% viscosity change per sec.)	Inhibition, %
Uninhibited	126.8	103.7	.073	91
Polymer I, 240 μg/ml	133.2	131.1	.0064	
Uninhibited	122.6	98.3	.078	92
Polymer II, 250 μg/ml	125.0	123.0	.0064	
Uninhibited	125.2	107.1	.056	92
Polymer III, 240 μg/ml	132.0	130.5	.0046	
Inhibition of lysozyme (.2 μg/ml) at pH 5.28				
(1) Substrate + saline	125.6	125.1	.0016	
(2) Substrate + polymer I (1.9 μg/ml) + saline	125.6	124.5	.0034	
(3) Uninhibited	124.4	106.4	.0580	
(4) Polymer I (.48 μg/ml)	132.4	119.5	.0390	37*
(5) " (1.9 μg/ml)	132.6	125.0	.0230	65*
(1) Same as (1) above				
(2) Substrate + polymer III (1.9 μg/ml) + saline	155.0	154.5	.0012	
(3) Uninhibited	126.6	117.2	.0266	
(4) Polymer III (1.9 μg/ml)	137.6	131.1	.0190	38*

* Rates of enzymatic reactions were corrected by subtraction of spontaneous (non-enzymatic) viscosity change, before % inhibition was computed.

$$\text{Rate} = \%\text{ change in SV/second} = \frac{(t_o - t_T) \cdot 100}{(t_o - t_{H_2O}) \cdot 500}$$

where t_T and t_o are the outflow intervals at time T and zero, respectively. The values for t_T and t_o are obtained by means of equation (1) from the SV-values taken by interpolation and extrapolation, respectively, from the specific viscosity-time plots. The rates allow computation of per cent inhibition values, which are presented in Table I.

Scarcity of substrate made necessary the following reasonable assumptions. (a) The substrate does not undergo a viscosity change at pH 2.15 in presence of polymers II or III, although this was demonstrated only for polymer I. (b) The spontaneous viscosity decrease at pH 5.3 determined in presence of polymer I is applicable to polymer III. (c) The effect of inhibitor concentrations on rate of spontaneous (non-enzymatic) viscosity decrease is negligible.

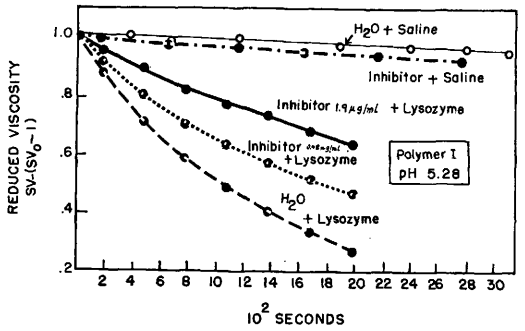

FIG. 3. Inhibition of lysozyme (0.2 μg/ml) action by polymer I at pH 5.28.

(B) *Peptic digestion.* Results of the pepsin experiments appear in Table II.

Discussion. Since only polymer I gave a positive effect in Shay rats the all-or-nothing effect noted in the animal experiments is not reflected by a similar, sharp delineation of anti-enzyme potencies: each of the polymers tested inhibited one, or both, of the enzymes. However, at the 250 mg level inhibition of pepsin caused by polymers II and IV is only about 50%, compared to 80% for polymer I. No difference was seen among the 3 polymers tested at pH 2 against lysozyme but at pH 5.2 again polymer I gives greater inhibition than polymer III. Conceivably such a difference might also have been observed at pH 2 if lower inhibitor concentrations were employed, but exhaustion of the substrate prevented us from making such measurements.

Thus, the *in vivo* experiments can be related to at least a trend in the anti-enzyme behavior of the compounds. If the inhibitory power of the polymers with regard to pepsin and/or lysozyme is, indeed, connected with their anti-ulcer potency, then the relatively small differences observed in the present study

TABLE II. Inhibition of Peptic Digestion of Coagulated Egg White in Mett Tubes. Pepsin: 250 μg/ml in 0.056 N HCl. Incubation: 16-20 hr at 37°. d = length of egg white column digested.

		Polymer I			Polymer II			Polymer IV		
Inhibitor, μg/ml	None	500	250	125	500	250	125	500	250	125
d^2	2450	121	529	1764	169	1296	1681	121	1089	1521
	2500	121	676		1369	1369	2116	121	1156	1849
	1764	100	289	1190	812	812	1296	144	900	1521
	2304	121	504	1480						
% inhibition avg	0	95	81	32	95	49	24	94	53	27

might lead one to search for more powerful anti-pepsins, or anti-lysozymes in the hope of uncovering therapeutically more valuable compounds.

Summary. (1) Ability of 4 acidic polymers to inhibit lysozyme and/or pepsin has been measured. (2) Their anti-enzyme effect is compared to their action in the Shay rat.

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Force-Velocity Relationship of Tetanus Toxin Treated Skeletal Muscle.* (24600)

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The mechanical properties of muscles actively shortened by tetanus toxin injection vary from normal in a manner and to a degree not solely attributable to atrophy(1). These muscles exhibit increased tetanus-twitch ratio, shortened twitch relaxation time, as well as lower fusion frequency(2), and an augmented semi-dynamic stiffness, at short degrees of stretch, following bouts of tetanic stimulation(3). Such observations are not readily interpreted when considered as isolated facets of the mechanical behavior of such actively shortened muscle. To extend

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