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Partially Depolymerized Hyaluronic Acid (PDHA) as a Spreading Agent. (20818)

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The spreading action of testicular hyaluronidase is usually attributed to the decrease in viscosity of the ground substance which results from depolymerization of the hyaluronate by the enzyme. The effect is to decrease the normal resistance of the barrier. A more recent view suggests that the enzyme releases PDHA which as a protective colloid and peptizing agent participates actively in the dispersion and transfer of materials through the ground substance and in the urine(1). It has also been suggested that hyaluronic acid is capable of acting as an ion exchange resin(2). In this paper we present data on the spreading properties of PDHA prepared from *in vitro* hydrolysis of streptococcal hyaluronic acid by testicular hyaluronidase.

Materials and methods. Bacterial hyaluronic acid was isolated by Dr. George H. Warren from a virulent strain of group A *Streptococcus pyogenes* obtained from the Hospital of the Rockefeller Institute. It contained 3.67% N and was free from S, P, and halogen. Purified bovine vitreous humor hyaluronate and human umbilical cord hyaluronate were prepared by Dr. Harvey E. Alburn. Streptococcal hyaluronate before treatment with the enzyme was the most viscous and vitreous humor hyaluronate the least viscous preparation. PDHA was prepared by incubating streptococcal hyaluronic acid with testicular hyaluronidase* for 15 minutes at 38°C. The incubation mixture was autoclaved for forty minutes at 104°C and the resulting precipitate removed by centrifugation and discarded. The supernatant was found to be free from hyaluronidase by the turbidimetric assay and therefore contained less than 0.1 TRU/ml. Incubation of the substrate with the enzyme for one hour resulted in supernatants that had no significant spreading activity. Spreading tests were made on albino rabbits of both sexes weighing between 2.3 kg and 2.9 kg. The intradermal injections were made through a 26 gauge, one-half inch needle into the lumbar and scapular regions clipped free from hair. The injections consisted of 0.1 ml of 1% trypan blue in physiological salt solution (PSS) mixed with 0.1 ml of test solution. Measurements of the large diameter of spread (D) and the small diameter of spread (d) were made with vernier caliper immediately after injection and 15, 30, and 60 minutes later. The area of spread was computed from the formula for the area of an ellipse, $A_{sq\ mm} = \frac{D \times d \times \pi}{4}$. Each concentration of a substance was tested on a minimum of 6 rabbits. Each rabbit received a control injection of PSS. The injection sites for the control and materials to be tested were randomized. The comparative efficiency of hyaluronidase and PDHA to facilitate hypodermoclysis of PSS was carried out in rabbits. Six rabbits received

* Wydase®—Wyeth Laboratories, Inc.—7000TRU/mg N.

TABLE I. Effect of Increasing Concentrations of Hyaluronidase on the Spreading Reaction.

	Area of spread in 60 min. (mm²)					
TRU inj.	100	1000	2000	5000	10000	20000
Mean	197.2	388.2	483.0	643.6	675.6	587.2
± SD %	9.2	4.8	10.6	4.4	2.8	5.4
No. of rabbits	6	8	6	10	6	6

100 ml of PSS in one flank and 100 ml of PSS containing 150 TRU of hyaluronidase in the other. Six rabbits received 100 ml of PSS in one flank and 100 ml of PSS plus 3 ml of 5% PDHA in the other. A comparison of the ability of hyaluronidase and PDHA to facilitate the spread and absorption of an x-ray contrast medium was made in female guinea pigs. Eight guinea pigs received 1 ml of Urokon® sodium† (Mallinckrodt) mixed with 3 ml of PSS subcutaneously over the lumbar region. Eight guinea pigs received 1 ml of the contrast medium plus 3 ml of PSS containing 150 TRU of hyaluronidase. Eight guinea pigs received 1 ml of the contrast medium plus 3 ml of 5% PDHA. Serial x-rays were taken at 5 minute intervals and the area of spread of the contrast medium was measured with a planimeter. The data were analyzed statistically for homogeneity within groups and for significant differences between groups by analysis of variance for paired and unpaired data.

Results. The mean area of spread in the 78 control injections consisting of PSS and trypan blue was 153.5 sq mm with a standard error of ± 5.25%. The effect of increasing concentrations of hyaluronidase on the area of spread is shown in Table I. It will be seen that increasing the concentration of enzyme from 0 to 10000 TRU per injection resulted in increasing area of spread. Further increase to 20000 TRU resulted in a sharp decline in spreading activity. It was not possible to inject larger amounts of the enzyme because of the viscosity of the solution. Plotting the logarithm of the turbidity reducing units against the mean area of spread results in a straight line with a slope of 2.34 from 100 to 10000 TRU.

† Sodium Acetrizoate 30% and 0.1% sodium citrate.

Spreading effects of hyaluronates from different sources. Streptococcal, umbilical cord, and vitreous humor sodium hyaluronates had no practical spreading action although statistically each of the substances exhibited some spreading at one concentration and an inhibiting effect at a higher concentration. Tested in groups of 6 to 8 rabbits in concentrations of 50, 100, 1000, 2000, and 5000 μg of sodium hyaluronate the streptococcal preparation gave respectively means (± SD%) of 147.1 (7.8), 217.2 (14.4), 198.1 (9.3), 163.7 (15.9), and 100.3 (20.3) sq mm of spread in 60 minutes. The umbilical cord preparation gave respectively means of 160.7 (8.5), 193.5 (5.6), 202.2 (6.6), 149.5 (5.2), and 112.9 (6.9) sq mm of spread in 60 minutes. The vitreous humor preparation gave respectively means of 202.2 (6.8), 194.7 (15.1), 171.5 (12.2), 142.3 (11.8), and 138.3 (4.1) sq mm of spread in 60 minutes. The spreading action was not inversely related to viscosity of the preparation. Streptococcal hyaluronate which gave the most viscous solutions had the greatest spreading action whereas the vitreous humor hyaluronate which gave the least viscous solutions had the least spreading action. Further evidence that the viscosity alone was not the determinant in the spreading action is seen from the fact that the lowest concentration was not always the most effective spreading concentration. Other polymers such as degraded alginic acid, undegraded pectin, and clinical grade dextran tested in identical fashion and in identical concentrations showed no spreading activity statistically.

Spreading action of PDHA. From Table II it will be seen that PDHA had considerable spreading activity which almost equalled that produced by 2000 TRU of hyaluronidase. It will also be seen that more completely hydro-

TABLE II. Effect of Depolymerized Sodium Hyaluronate (Streptococcal) on the Spreading Reaction.

	Area of spread in 60 min. (mm²)	
	⌜—Enzymatic hydrolysis—⌝	
	15 min.	1 hr
	5 mg inj.	5 mg inj.
No. of rabbits	12	6
Mean	553.1	241.3
± SD %	10.6	12.4

TABLE III. Effect of Hyaluronidase and PDHA on Hypodermoclysis in Rabbits. 6 rabbits in each series.

	Time for hypodermoclysis (min.)			
	Saline	Hyaluronidase (150 TRU)	Saline	PDHA (150 mg)
Mean	47	19	51	18
± SD %	7.6	10.2	8.4	12.7

TABLE IV. Effect of Hyaluronidase and PDHA on Absorption and Excretion of Urokon.

Treatment group*	Spread, mm² at time of inj.		Appearance in urinary bladder (min.)
Saline + Urokon	Mean	4.4	30
	± SD %	22.7	
150 TRU hyaluronidase + Urokon	Mean	10.4	16.3
	± SD %	16.3	
150 mg PDHA + Urokon	Mean	11.4	16.9
	± SD %	30.7	

* 8 guinea pigs/group.

lyzed hyaluronate was without significant spreading activity.

Hypodermoclysis and absorption of x-ray contrast medium. The ability of PDHA to facilitate a hypodermoclysis of PSS in rabbits is shown in Table III. It will be seen that 150 mg of PDHA was as effective as 150 TRU of the enzyme. In other experiments 50 and 100 mg of PDHA added to the clysis was without facilitating effect. Facilitation of spread and absorption of an x-ray contrast medium in guinea pigs is shown in Table IV. It can be seen that 150 TRU of hyaluronidase or 150 mg of PDHA increased the area of spread and the rate of absorption and appearance of the opaque material in the urinary bladder by twofold.

Summary. 1. Increasing the concentration of hyaluronidase by large units resulted in increased spreading activity until a massive concentration of enzyme was used. 2. Highly polymerized hyaluronic acid obtained from *Streptococcus pyogenes,* bovine vitreous humor, and human umbilical cord had no significant spreading action. 3. Partially depolymerized streptococcal hyaluronic acid had significant spreading action in rabbits, hastened the absorption of a clysis of physiological salt solution in rabbits and facilitated the spread and absorption of an x-ray contrast medium in guinea pigs. 4. Further depolymerization of the streptococcal hyaluronic acid resulted in products that no longer had significant spreading action.

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Topical Protection Against Hyperergic Inflammations by a Preceding Local Inflammation. (20819)

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The hyperergic reaction provoked in the rat by the intraperitoneal injection of fresh egg albumin(1) can be topically inhibited by means of a previously produced local inflammation. This was demonstrated by Selye and his collaborators(2), who stated that the degree of topical protection depends on the intensity of the preceding inflammation and considered the phenomenon as not specific, since various irritative substances inhibit topically the hyperergic reaction produced by the egg albumin injection. As regards the mechanism of this protecting action, the aforementioned authors assert that it is strictly locally conditioned.

Method. Fifty white 4-months-old rats were used, with a body weight of between 100 and 130 g, distributed in 5 groups of 10 rats