both absorbed 90% of the light. Both solutions were then reduced with sodium hydrosulphite and the light absorption values again determined. The reduced dopa melanin absorbed 66% of the light, while the reduced squid melanin absorbed 76% of the light. The melanin solutions were then re-oxidized with potassium ferricyanide. The re-oxidized dopa melanin absorbed 89% of the light, while the re-oxidized squid melanin absorbed 88.5%. This experiment was repeated 22 times with ink from 35 squid. The results were always uniform.

It may be seen from the light absorption values that the reversibility of the oxidation of the natural squid melanin approached that of synthetic dopa melanin. The figures indicate that the squid melanin was contaminated with some substance that absorbed about 10% of the light and which was not reversibly oxidizable. This substance may have been melanin that had aged in the squid ink-sac because even synthetic melanin 6 months old was not reversibly oxidizable.

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Relationship between "Spreading Factor" and Hyaluronidase.*

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Duran-Reynals[1] demonstrated the presence of an extractable factor in certain tissues and bacteria which enhances the invasiveness of some pathogenic agents. This factor has been extensively studied by Duran-Reynals and by others.[2, 3] Chain and Duthie[4] reported that testis extracts containing "spreading factor" decrease the viscosities of synovial fluid and vitreous humor with the liberation of reducing substances. They suggested that "spreading factor"

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1 Duran-Reynals, F., *Compt. rend. Soc. biol.*, 1928, **99**, 6; *J. Exp. Med.*, 1929, **50**, 327; *ibid.*, 1933, **58**, 161; *Yale J. Biol. and Med.*, 1939, **11**, 601.

2 McClean, D., *J. Path. and Bact.*, 1930, **33**, 1045; *ibid.*, 1936, **42**, 477.

3 Claude, A., *J. Exp. Med.*, 1937, **66**, 353; Claude, A., and Duran-Reynals, F., *J. Exp. Med.*, 1937, **65**, 661.

4 Chain, E., and Duthie, E. S., *Nature*, 1939, **144**, 977.

is probably identical with the "mucinase" which hydrolyzes the polysaccharide in these fluids.

A mucopolysaccharide designated as hyaluronic acid has been isolated from vitreous humor, unbilical cord,[5] the mucoid phase of Group A hemolytic streptococci,[6] synovial fluid,[7] fowl sarcoma[8] and the pleural fluid of a patient with a mesothelioma.[9] Enzymes which hydrolyze hyaluronic acid have been prepared from pneumococci, Group A hemolytic streptococci, *Cl. welchii,* and splenic tissue.[10] More recently Meyer and Chaffee[11] confirmed the observation of Chain and Duthie and demonstrated that testis extracts hydrolyze isolated hyaluronic acid as well as the polysaccharide acid of cornea. The presence of hyaluronidase in high concentration has also been demonstrated in leech extract.[12]

The present study was undertaken to determine more precisely the relationship between "spreading factor" and hyaluronidase. The spreading capacity of the various preparations was tested by the intracutaneous method (using T.1824).[1] The action of hyaluronidase was tested (1) by the hydrolysis of isolated hyaluronic acid and (2) by determining changes in the viscosity of various fluids known to contain hyaluronic acid.†

The presence of hyaluronidase and "spreading factor" was tested in preparations from the following sources: pneumococci (Type I virulent and avirulent strains, Type II avirulent strain), Group A hemolytic streptococci (virulent and avirulent strains in the mucoid and rough phases), *Cl. welchii,* testis, pigskin, leech extract, commercial hirudine, and certain chemical substances.

Results. All preparations containing hyaluronidase were found to possess spreading properties. In addition hyaluronidase and "spreading factor" were found to possess certain attributes in common. The activity of both was weakened by heating at 65°C for

5 Meyer, K., and Palmer, J. W., *J. Biol. Chem.*, 1936, **114**, 689.

6 Kendall, F. E., Heidelberger, M., and Dawson, M. H., *J. Biol. Chem.*, 1937, **118**, 61.

7 Meyer, K., Smyth, E. M., and Dawson, M. H., *J. Biol. Chem.*, 1939, **128**, 319.

8 Kabat, E. A., *J. Biol. Chem.*, 1939, **130**, 143.

9 Meyer, K., and Chaffee, E., *J. Biol. Chem.* 1940, **133**, 83.

10 Meyer, K., Hobby, G. L., Chaffee, E., and Dawson, M. H., *J. Exp. Med.*, 1940, **71**, 137.

11 Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 487.

12 Claude, A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 684. Unpublished data.

† The assumption has been made by some investigators that capacity to reduce viscosity constitutes a test for the presence of "mucinase." While it is apparently true that hydrolysis and decrease in viscosity are catalyzed by the same agents, it is not certain that the two reactions are due to the same enzyme.

30 minutes and destroyed at 100°C; both were destroyed or markedly weakened by iodine and, under the experimental conditions, neither was reactivated by sodium sulfite.

The theory that spreading factor and hyaluronidase are identical postulates the existence in skin of either hyaluronic acid or a similar substrate on which hyaluronidase may act. Recently, in this laboratory, a polysaccharide acid which is hydrolyzed by hyaluronidase preparations has been isolated from skin. The nature of this polysaccharide will be described elsewhere.

The evidence so far presented suggests that spreading factor may owe its action to the presence of hyaluronidase. On the other hand, certain observations suggest that the two are not identical.

A number of preparations which possessed marked spreading action did not hydrolyze hyaluronic acid nor did they reduce the viscosity of solutions containing the polysaccharide. Among these were several preparations from different strains of Group A hemolytic streptococci, pigskin, commercial hirudine, arsenious oxide and hyaluronic acid.‡

A further possible point of distinction was observed between hyaluronidase and "spreading factor." Antiserum made against hyaluronidase prepared from pneumococci specifically and completely inhibited the activity of the homologous enzyme but did not inhibit the spreading action of the pneumococcal preparations. This difference may be due to the combination of pneumococcal hyaluronidase with the antiserum to form a loose complex which may be inactive *in vitro* but may dissociate to an active form *in vivo*. It should be mentioned, however, that the antiserum to the pneumococcal enzyme did not inhibit the action of hyaluronidase prepared from streptococci nor did it affect the spreading action of such preparations.

Summary. Evidence is presented to show that hyaluronidase and "spreading factor" exhibit certain attributes in common. However, there is also considerable evidence that "spreading factor" does not owe its activity solely to the presence of hyaluronidase. It would seem probable that the phenomenon of "spreading" is a complex one and that several factors may be involved in its production. Further work is required to explain the mechanism of "spreading" in terms of known chemical and physico-chemical reactions.

‡ Duran-Reynals[1] has also shown that skin contains spreading factor. The spreading effect produced by simple chemical substances may be due to the release of "spreading factor" locally at the site of injection.