sionally results in the formation of several chromatic clumps, apparently representing unequal groups of chromosomes (Figs. 1 and 2). These groups may sometimes be found still connected to each other by chromatic strands.

Cycloheximide, like streptomycin, is an antibiotic produced by *Streptomyces griseus,* and has marked fungicidal properties. Whiffen (*4*) recently showed that the treatment of the sporophytes of *Allomyces arbuscula* with cycloheximide in very low concentrations resulted in the development of gametophytic outgrowths, thus indicating somatic reduction. However, this effect was observed only after 10–14 days, in contrast to the early observable effects upon the basidia of *Gymnosporangium.* According to Wilson (*5*), the substance has been shown to have interesting cytological effects in onion-root tips, in which it may induce the formation of "reductional groupings" of chromosomes during somatic mitosis. In our work with *Gymnosporangium,* the effective range of concentrations of cycloheximide has been found to be between 5–500 ppm. At the higher concentrations basidial development is almost completely inhibited and the nuclei become very large, with much extended chromosomes. Cyclohèximide also causes a forking of basidia similar to that induced by penicillin, and in some instances there occurs a lagging of the chromosomes during meiosis. Lower concentrations cause a return of one of the nuclei to the teliospore, as was found in penicillin- and streptomycin-treated material. The most striking effect of cycloheximide is observed in the occurrence of many basidia with 2 nuclei closely associated, or still connected to each other by chromatic strands, in sharp contrast to the usual long distance between daughter nuclei following anaphase. This may be due to malfunction or disintegration of the spindle and might result occasionally in the reconstitution of a single diploid nucleus rather than two haploid ones.

References

1. COLLEY, R. H. *J. Agr. Research,* **15**, 619 (1918).
2. OLIVE, L. S. *J. Elisha Mitchell Sci. Soc.,* **58**, 43 (1942).
3. ———. *Amer. J. Bot.,* **36**, 41 (1949).
4. WHIFFEN, A. J. *Mycologia,* **43**, 635 (1951).
5. WILSON, G. B. *J. Hered.,* **42**, 251 (1951).

Manuscript received November 12, 1952.

Effects of Hyaluronidase on Human Gingival Epithelium[1]

S. Schultz-Haudt, M. Dewar, and B. G. Bibby

Eastman Dental Dispensary, Rochester, New York

As part of a study of the mechanism by which accumulations of gingival bacteria produce marginal inflammation, we were able to demonstrate the presence of a "spreading factor" in cell-free extracts of gingival debris. This fact, together with the known presence of the enzyme hyaluronidase in saliva (*1*) and

[1] Supported by U. S. Army Grant No. DA-49-007-MD-159.

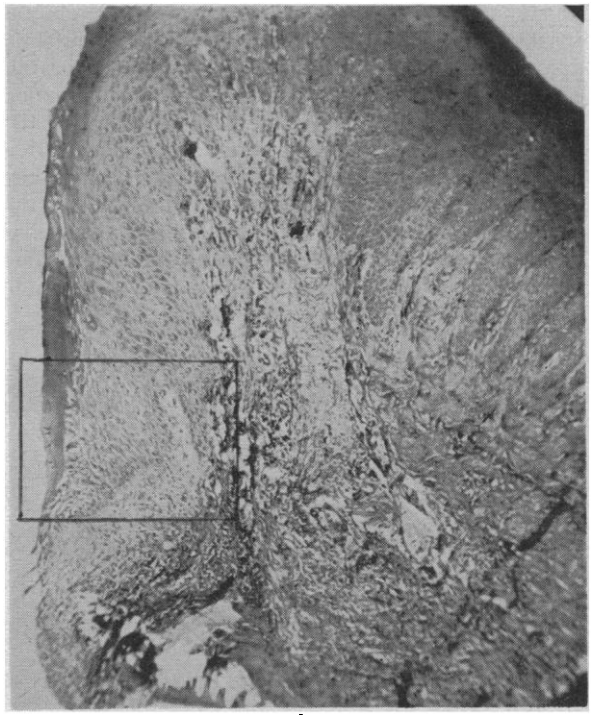

FIG. 1. Gingival tissue after 2 days of treatment with hyaluronidase. Paraffin embedding; section thickness 6 μ; stained with Masson's Trichrome stain. Area of dissolution of the pocket epithelium indicated. The connective tissue presents an irregular appearance.

the demonstration of high production of hyaluronidase by certain oral bacteria (*2*), made it seem important to examine the effect of this enzyme applied topically on human gingival tissues. The connective tissue reactions stimulated by hyaluronidase have been described previously (*3, 4*). However, these descriptions do not discuss the changes brought about in the epithelium or the manner by which the enzyme can reach the underlying connective tissue. Apart from general interest, this problem has special importance in gingivitis because it is not known whether the hyaluronidase reaches the connective tissue as the result of trauma, through a weak epithelial attachment, or by passage through the epithelium itself.

Biopsies of human gingiva exposed to the action of hyaluronidase (Wydase) were chosen as a means of determining the tissue reactions. Both experimental and control tissues were taken from each of 36 individuals' normal and inflamed gingiva. The hyaluronidase solution was always used in a fresh state as 150 TR.U./cc in sterile triple-distilled water. It was introduced into the gingival crevice by a small syringe; extreme care was taken to avoid injury to the tissue. The delivery rate was about 12 TR.U./min. When inactivated hyaluronidase was used, the enzyme was either inactivated by heat or by addition of heparin to the solution. After the experimental period, the treated gingival tissues were carefully removed by biopsy. After fixation in Carnoy's fluid the tissues obtained were embedded in paraffin and sectioned. A

variety of staining procedures was employed: Harris' hematoxylin and eosin; the Masson Trichrome stain for registration of epithelial changes; the Gomori silver strain and the Weigert fuchselin-GG combination for demonstration of elements of the connective tissue; and the Gram-Weigert strain for demonstration of Gram-positive microorganisms in tissues. Two histochemical techniques were carried out routinely: the Hotchkiss procedure for demonstration of polysaccharide-containing materials in tissues, and the Hale staining techniques for demonstration of hyaluronic acid in tissues.

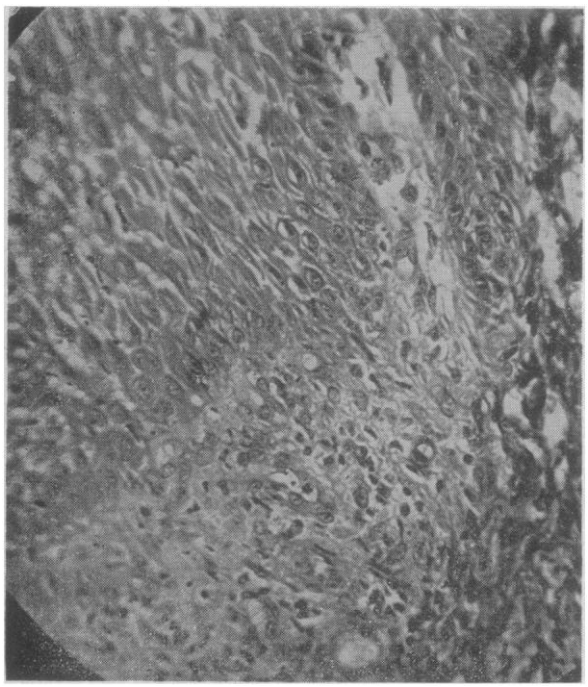

FIG. 2. Higher magnification of framed area of Fig. 1 showing destruction of cell bridges and removal of intercellular substance.

Histological examination of the Harris and Masson stained biopsy tissues showed that in the tests lasting less than 1½ days, the epithelium seemed unaffected by the enzyme, but dilated vessels and an appearance of loosening of the typical connective tissue structure indicated an effect in the subjacent tissues. Hotchkiss-stained epithelium consistently showed an increased amount of glycoprotein after a few hours of hyaluronidase action. Variations in the tissue reaction in relation to the enzyme concentration used or the duration of exposure to the enzyme were more marked than variations between individuals.

In 8 out of 9 tests lasting more than 1½ days some alteration of the epithelium was noted, and the changes in the connective tissue were marked. Figure 1 shows a typical "loosening up" of the epithelium, and Fig. 2 a higher power magnification of the area in question. Figure 2 reveals an actual spacing between the epithelial cells resulting from solution or destruction of cell-bridges and intercellular substance. The connective tissue presents an irregular appearance with large vacuoles and dilated vessels. Another irregularity that indicates changes in the epithelium was revealed by the Masson stain, which produced purple-to-bluish colored areas in the epithelial tissue instead of the usual bright red coloration. In marked contrast to the control sections (Fig. 3), the Hotchkiss stain demonstrated a rather large increase of stainable polysaccharide material within the epithelial layers of the experimental area (Fig. 4).

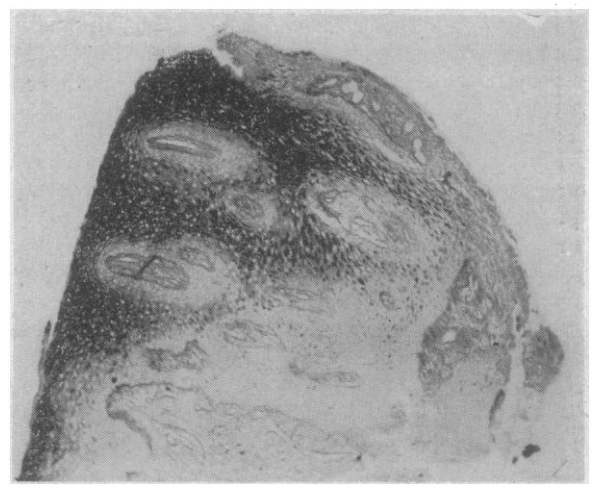

FIG. 3. Gingival tissues treated with hyaluronidase (2 hr); embedded in paraffin; section thickness 6 μ stained according to Hotchkiss technique. Polysaccharide materials show as black areas within the epithelium.

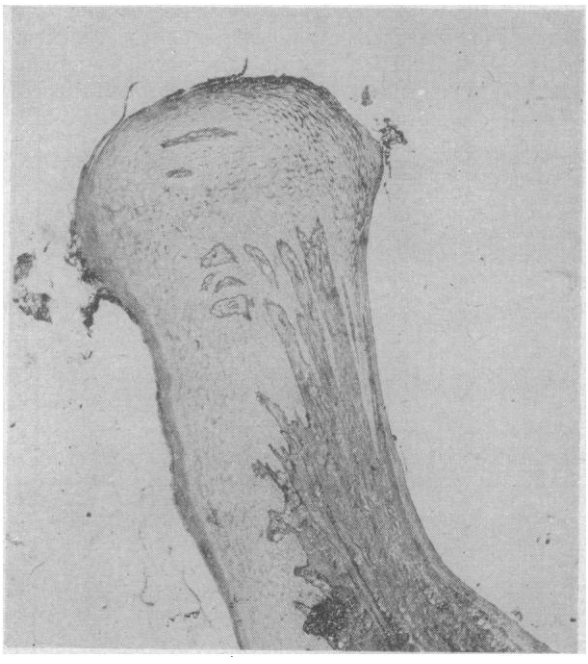

FIG. 4. Clinically normal gingiva; embedded in paraffin; section thickness 6 μ, stained according to Hotchkiss technique. Small areas of polysaccharide materials appear black within the superficial layers of the marginal epithelium.

It may be concluded that the initial effects of hyaluronidase on epithelium are an increase in polysaccharide-containing materials and prolonged action which is capable of altering the intercellular substance in the gingival epithelium, thereby permitting passage of destructive agents to the underlying connective tissues. In the connective tissue, hyaluronidase produces dilated vessels, vacuolation, and breakdown of the typical structure.

On the basis of these findings, it is suggested that various hyaluronidase-like products of gingival bacteria may produce similar changes in the gingiva and thus contribute to gingivitis.

References

1. LISANTI, V. F. *J. Dental Research,* **29**, 392 (1950).
2. ———. Personal Communication.
3. AISENBERG, M., and AISENBERG, A. *Oral Surg., Oral Med., Oral Pathol.*, **4**, 317 (1951).
4. GERSH, I., and CATCHPOLE, H. R. *Am. J. Anat.*, **85**, 457 (1949).

Manuscript received October 30, 1952.

Reversible Reaction of Chlorophyll Giving the Red-Brown Intermediate of the Molisch Phase Test[1]

Simon Freed and Kenneth M. Sancier

Department of Chemistry,
Brookhaven National Laboratory,
Upton, Long Island, New York

Chlorophyll[2] *b* or *b'* which had its solvent removed at 0° C in a vacuum line was covered with isopropyl amine by condensation at the temperature of dry ice, and at once a red color appeared as the chlorophyll dissolved. The temperature was then raised gradually, and the color changed, acquiring a greenish tint, becoming red-brown, green-brown, and finally green. At about 230° K, the spectrum approached the standard spectrum of chlorophyll (*1*) *b* or *b'*. When the temperature was then lowered, the change in color followed in reverse order. However, if the solution had been kept any length of time at the higher temperature, the original intensity of the red was not fully restored. An irreversible reaction had transformed the chlorophyll into another substance as the spectrum confirmed at the higher temperature.

Figure 1 shows the spectra of chlorophyll *b'* as they change with temperature. The chlorophyll was dissolved in 10% mono-isopropyl amine in 1:1 propane-propene. Figure 2 gives the spectra of chlorophyll *a* dissolved in 10% isopropyl amine, 10% isopropyl benzene, and the remainder 1:1 propane-propene. When the amine was diluted, as in the above solutions, the extent of the irreversible reaction was unimportant even at the highest temperature we are considering.

Our identification of the colored intermediate substances with those of the Molisch phase test rests on the spectra obtained by Dunicz *et al.* (*2*) at the confluence of two streams at room temperature, one consisting of chlorophyll in ether and the other trimethyl benzyl ammonium hydroxide in methanol. This technique had been devised to cope with the fact that the brown color disappeared in a fraction of a second at room temperature. The finely dotted curve in Fig. 2

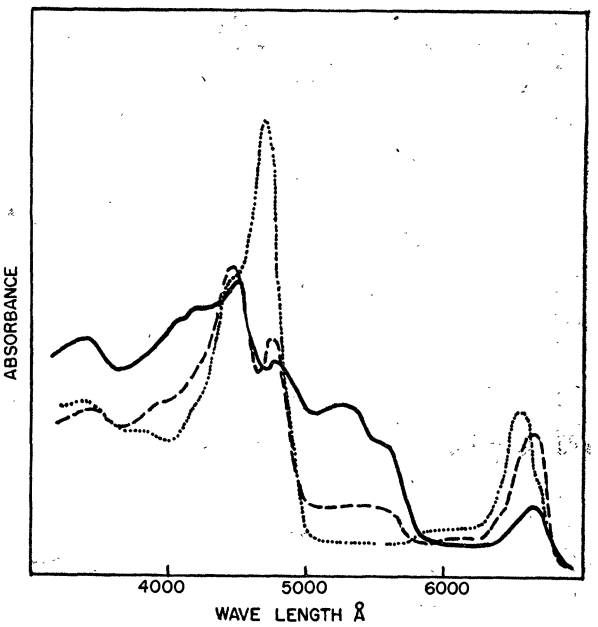

FIG. 1. Chlorophyll *b'* in 10% mono-isopropyl amine and 1:1 propane-propene, ... 230° K, – – – – – 193° K, —— 160° K.

gives the absorption spectrum of the brown intermediate of chlorophyll *a* when correction has been applied for the presence of chlorophyll as well as of the end product of the irreversible reaction. The similarity in the structure of this absorption with that obtained at the lowest temperature in Fig. 2 is evident, especially so in the strong absorption in the green region 4500 to 5500 A. Also should be noted the simultaneous decrease with decreasing temperature in the intensities of the characteristic absorption peaks of chlorophyll in the red and in the blue.

When the isopropyl amine was diluted with hydrocarbons, a lower temperature was required to match the intensity of the color that was obtained when the solvent consisted of pure amine. Since the formation of the intermediate depended on the concentration of the base, the reaction was at least bimolecular. As confirmation may be offered the discovery that the green solution was frozen-in by sudden quenching to the temperature of liquid nitrogen even though the solution still remained fluid. On allowing the temperature to rise gradually, very little change in color was ob-

[1] Research carried out under the auspices of the U. S. Atomic Energy Commission.

[2] Solutions of purified chlorophylls *a*, *b*, and *b'* and of allomerized chlorophyll *a* in ether were generously furnished us by Robert Livingston and his associates at the University of Minnesota. ONR Project N60 ri-212 Task Order 1.