

Enzymic Degradation of Cellulose Fibers

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Introduction

It has long been known that when fungi degrade cotton, the tensile strength decreases at a rapid rate. Other properties (except a few noted under the microscope), however, change very little or not at all. Such observations have led to the conclusion that microbiological degradation of cotton is, to a very large extent, a localized process [3]. The fundamental question immediately follows: In which region or regions of the fiber does the destructive attack occur? An answer to this question would be of importance in clarifying the general problem of microbiological degradation of cotton, and would perhaps assist in finding better means of preventing or retarding such degradation. Accordingly, the work reported in this paper was undertaken in an attempt to supplement the existing knowledge in this field.

Much of the work published on this subject had been carried out with the living microorganisms acting on the cotton. In the experiments described below, the effects of a *cell-free extracellular cellulolytic enzyme solution* on the properties of cotton

fiber and on two synthetic cellulose filaments were studied. A few comparative observations were also made on *fungus-degraded* cotton.

Experimental

Materials

A *cell-free extracellular cellulase solution* was prepared by the method of Saunders, Siu, and Genest [17] from the fungus *Myrothecium verrucaria* (QM 460), except that the following nutrient salt solution was used: NH_4NO_3 , 3.00 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.22 g.; KH_2PO_4 , 2.59 g.; K_2HPO_4 , 2.21 g.; H_2O , 1l. The enzyme filtrate was preserved under toluene.

Cotton fibers in the form of sliver were purified by extracting with ethyl alcohol in a Soxhlet extractor for 8 hrs., and then with ethyl ether for 2 hrs. After drying at 50°C the fibers were refluxed in 1% sodium hydroxide under nitrogen for 7 hrs., and washed free of caustic with tap water, dilute hydrochloric acid, and distilled water. The material was finally dried at 50°C overnight.

Viscose rayon strands* known as "regular bright" and cuprammonium (Bemberg) rayon* known as "Grade No. 1" were extracted with alcohol and ether and dried at room temperature.

Specimens of fungal-degraded cotton duck, which were incubated for 14 days with *Alternaria* sp., *Fusarium diversisporum*, *Cladosporium herbarum*, *Curvularia lunata*, *Epicoccum* sp., or *Chaetomium globosum*,† were studied. These specimens had lost from 79%–92% of their original breaking strength.

Methods

Preparation of Samples.—Accurately weighed samples of each of the three materials (approximately 8 g. of cotton, 15 g. of viscose rayon, and 7 g. of Bemberg rayon) were incubated in closed flasks with 500 ml. of the enzyme solution at 40°C for varying time periods. A parallel series of samples as controls were incubated with the basic salt solution alone. At the end of each incubation period the samples were washed by decantation with about 15 portions of distilled water and partially dried at 50°C. Drying was completed in a vacuum oven at 50°C. A third series of flasks containing enzyme alone was allowed to remain at 40°C for varying time periods in order to estimate the rate of loss of enzyme activity.

In an early exploratory experiment, cotton samples were prepared as described above, except that they were incubated at 23°C for 27 days.

Before decantation, tests were made for bacterial and fungal contamination by inoculating tubes of peptone agar and potato dextrose agar with the solutions. The results were all negative.

Chemical and Physical Procedures.—The 1,4- β -polyglucosidase activity, reducing power, and pH of the enzyme solutions were determined before and after incubation. Activity was evaluated by measuring the change in the reducing power of hydroxyethyl cellulose (HEC). With but minor modifications, the method is that described by Reese, Siu, and Levinson [15] for determining " C_x " activity. The reducing powers of the solutions after digestion of the fiber samples were determined by Sumner's procedure [21]. pH was measured directly with a glass electrode.

* These materials were kindly supplied by the American Viscose Corporation and American Bemberg Corp., respectively.

† These specimens were kindly supplied by Dr. E. T. Reese of the Mycology Section of the Pioneering Research Laboratories.

Immediately after drying, the fiber samples were reweighed in order to determine the loss of weight as a result of degradation. Changes in load-elongation properties, degree of polymerization, and degree of crystallinity were determined. Comparative microscopic observations were also made.

Load-elongation properties were determined with the Sookne-Harris single-fiber tester [19]. For cotton, the gage length was 1.3 cm. and the rate of elongation was 0.033 in. per min. For viscose rayon, the gage length was 4.3 cm. and the rate of elongation 0.2 in. per min. All samples were conditioned at 70°F and 65% relative humidity for a minimum of 3 days before testing. Because the Bemberg rayon lost almost all of its strength very rapidly, it was not possible to separate filament samples for load-elongation determinations.

Degree of polymerization (D.P.) was determined by the cupriethylenediamine hydroxide (cuprien) viscosity procedure described in A.S.T.M. Designation D 539-40T, and as developed by Conrad and Tripp [6, 7]. Specific viscosity, η_{sp} , was converted to intrinsic viscosity, $[\eta]$, by the equation of Tripp and Conrad [22]: $[\eta] = a\eta_{sp}^b - c$. For a concentration of 0.50 g./ml., $a = 1.70$, $b = 0.694$, $c = 0.160$; for a concentration of 0.25 g./ml., $a = 3.27$, $b = 0.786$, $c = 0.137$. Degree of polymerization was calculated using Staudinger's equation, $D.P. = K[\eta]$, with K taken as 190.

Changes in degree of crystallinity (D.C.) of enzyme-degraded fibers were determined from x-ray diffraction intensity tracings.* The ground samples were pressed into thin discs (approximately 0.8 mm.) to give essentially random distribution of the particle axis, thus eliminating the effect of orientation from the tracings. Diffraction intensities in the equatorial radial direction were recorded with the aid of the x-ray spectrometer and recorder. Similar traces were made in each case from a quartz surface, which served the purpose of calibration.

Microscopic Methods.—The samples were prepared for microscopic investigation in various ways. Most of the observations were made on fibers swollen in cuprien solution. This reagent, because of its greater stability, was found superior to cuprammonium hydroxide. By varying the strength of the solution, the rate and degree of swelling could be

* The x-ray diagrams were kindly prepared and interpreted by Dr. C. M. Conrad of the Southern Regional Research Laboratory, U. S. Dept. of Agriculture.

easily controlled and the specimens could be observed throughout the entire swelling process.

Specimens were cut into short lengths and dispersed in a drop of water on a slide. The water was then removed with absorbent paper and cuprien added either before covering with a glass slip or by irrigation after covering. By comparative observations with whole cotton fibers, it was found that cutting did not alter the characteristics of the swollen fibers. Cuprien solutions were prepared by diluting the solution used for viscosity determinations (2 parts ethylene diamine to 1 part copper, being 1M in copper). Dilutions of from 2 to 4 parts of cuprien to 20 parts of water by volume were found useful. Cotton specimens were also examined after swelling in sodium hydroxide and staining with Congo red according to the procedure of Clegg [5].

Photographs* were taken of cross sections of viscose rayon samples which had been differentially stained by the technique of Morehead and Sisson [13] to show the skin and the core. Electron photomicrographs of surface replicas of the viscose rayon were also made.

Results

Enzyme Activity

It has been postulated that more than one specific enzyme is involved in the degradation of cellulose. Pringsheim suggested [14] that one enzyme breaks the long-chain cellulose molecules to short chains (cellobiose), and another enzyme then hydrolyzes cellobiose to glucose. Reese and coworkers [12, 15] presented evidence that at least two enzymes, C_1 and C_2 , are involved in the breakdown of cellulose to cellobiose, with cellobiase hydrolyzing the latter compound to glucose. Hydrolysis of cellobiose may occur within the organism or extracellularly. In view of these considerations, it is possible that the measured activity against hydroxyethylcellulose is not a complete measure of the ability of the enzyme to degrade cellulose in the solid state. However, it is undoubtedly a good measure of the effective concentration of an important component of the cellulase system. The enzyme referred to in this paper is defined specifically by the method of preparation as described above, and its activity by the method of measurement.

* These photographs (Figures 11 to 14 inclusive) were taken by Dr. F. F. Morehead of the American Viscose Corporation. They were kindly released for publication by that company.

The results of the activity determinations are summarized in Figure 1. For the enzyme solution alone: at 5°C (storage temperature) there was no measurable loss in activity; at 23°C there was a gradual loss of activity with time; at 40°C the rate of loss of activity was greater. In the presence of cotton at 40°C the enzyme solution lost activity at about the same rate as the solution alone. Thus, the presence of cotton fiber appears to have had no significant effect on the activity of the enzyme solution. Loss in activity in this case, therefore, was very probably a temperature-time deactivation effect only. The activity curve for viscose rayon suggests the possibility that in the presence of this filament the enzyme lost activity at a slightly more rapid rate than did the enzyme alone. The deviations in the experimental data do not permit a rigid conclusion on this point. In contrast, the enzyme solution in the presence of Bemberg rayon lost activity very rapidly. This loss could not have been a temperature-time effect alone.

pH

Measurements of the original enzyme solution and of the solutions after incubation gave a pH of 6.0 \pm 0.1 in all cases.

Loss of Weight

Loss-of-weight measurements (Figure 2) showed a very low rate of loss for cotton (approximately 1% in 61 days), a somewhat faster rate for viscose rayon (2.1% in 39 days), and a very rapid rate (decreasing with time) for Bemberg rayon.

The loss of weight as calculated from the increase in reducing power of the solution gave consistently lower results than the direct measurements. Calculation of weight loss was based on the assumption that the portion of the cellulose which had been solubilized was changed completely to glucose. If the assumption were not true (*i.e.*, breakdown were not completely to glucose), the calculated weight loss would be less than the directly measured loss. It is indicated, therefore, that at least a certain portion of the soluble breakdown products was larger than glucose in size.

Degree of Polymerization

The degree of polymerization data are depicted graphically in Figure 3. The cotton and viscose rayon residues showed no significant change in D.P.

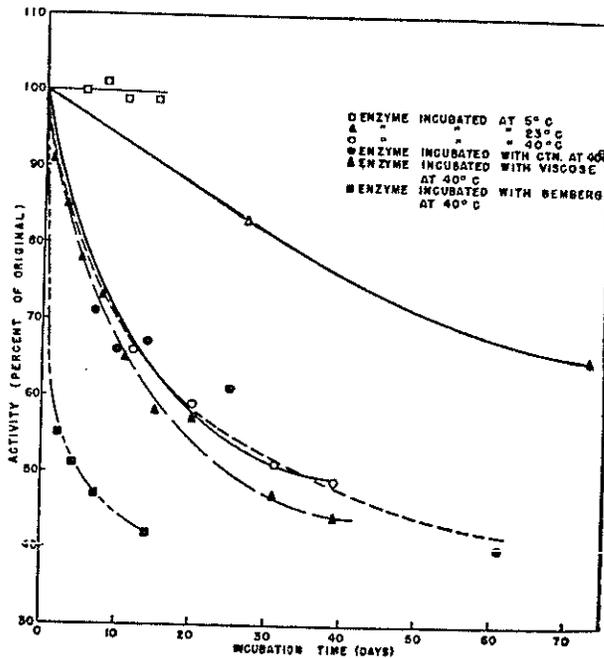


FIG. 1. Change in activity of enzyme solution with incubation time.

The small deviations are probably within the experimental error. Bemberg rayon, however, dropped very rapidly from a D.P. of 545 to a value of 141 in 3 days. Further changes with incubation time were insignificant.

Degree of Crystallinity

Interpretation of the x-ray data by Conrad indicated no essential changes in the degree of crystallinity of cotton digested for 3 and 61 days, as compared to the original cotton. There appeared to be a slight increase in crystalline diffraction over the control for viscose rayon digested for 11 and 39 days. Bemberg rayon samples which were digested for 2, 7, and 14 days showed a definite increase in crystalline diffraction over the control, but little or no differences in diffraction among the degraded samples.

Load-Elongation

The relationship between the loss of breaking strength (here defined as the load in grams at the break) and the elongation at break with incubation time is given in Figure 4. The control samples (incubated in salt solution alone) showed no significant deviation from the original samples and are therefore not included in the figure. The breaking-

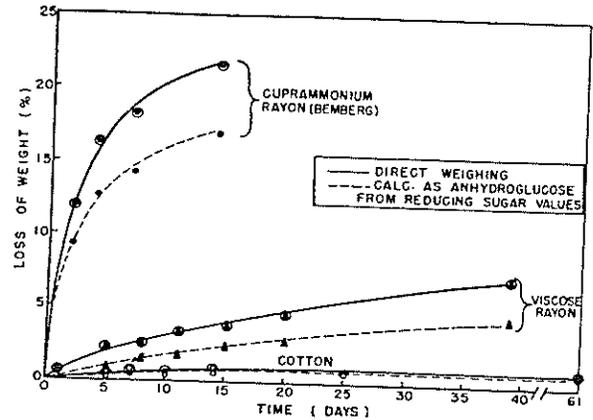


FIG. 2. Loss of weight of cellulose fibers with incubation time.

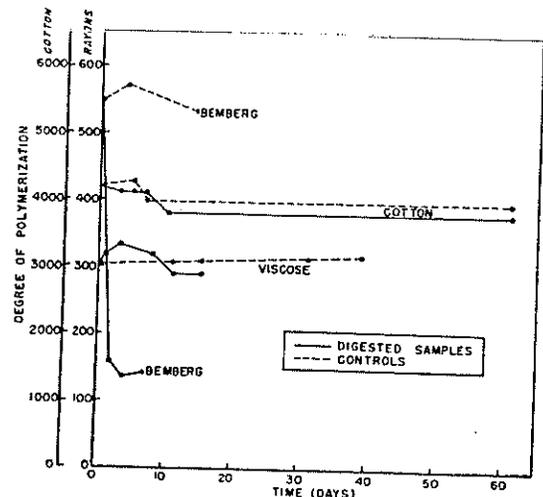


FIG. 3. Change in degree of polymerization of cellulose fibers with incubation time.

strength and elongation curves are similar, varying somewhat in magnitude. It appears, therefore, that enzymatic degradation affects these two properties in the same manner. For both viscose rayon and cotton, degradation (as measured by changes in these properties) proceeded at a very rapid and approximately equal rate during the first 3 days. Degradation of viscose rayon continued thereafter, but at a decreasing rate. Cotton in the presence of the enzyme solution at 40°C reached a maximum degradation of approximately 34% loss in breaking strength in about 3 to 5 days. At 23°C the extent to degradation of cotton (49% loss in breaking strength) was higher than at 40°C.

After 2 days of incubation with the enzyme solution, Bemberg rayon had apparently lost almost all

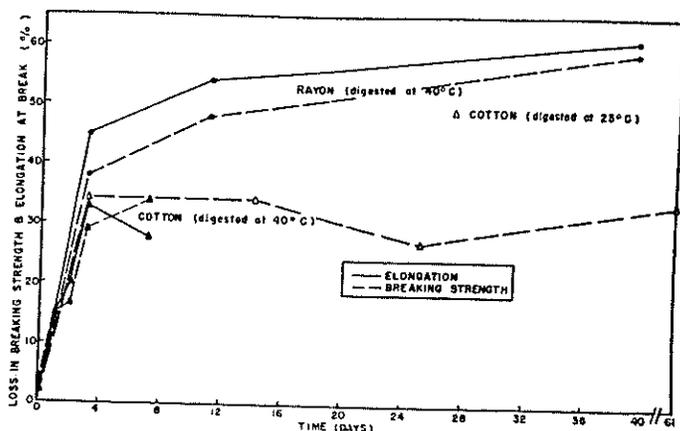


FIG. 4. Change in tensile properties of cellulose fibers with incubation time.

of its breaking strength. Only a very slight pull was required to break a strand of 74 filaments. They also crumbled readily under slight pressure.

Dye Absorption

When the fiber samples were prepared for microscopic observation by the Clegg procedure [5], it was noticed that degraded cotton and viscose were stained a much deeper red than the original materials. Both the degraded and undegraded Bemberg rayon stained very deeply and to approximately the same degree. The stained, degraded Bemberg samples had a slightly brown tinge.

Microscopic Observations

The observations of cotton fibers noted below are illustrated in essentials in Figures 5–10 inclusive.

The purified cotton as well as the salt control samples (Figure 5) swelled in cuprien in a manner reported many times in the literature (*e.g.*, Hock [10]). The spiral winding (the first layer of the secondary wall) was prominent, and appeared to restrict expansion generally over the entire fiber and also locally to cause ballooning. The type of ballooning caused by the formation of a narrow collar around the fiber by the primary wall or cuticle was observed, but was not very extensive as compared with unpurified fibers.

The enzyme-degraded samples differed in several respects from the original fibers. The spiral windings were not as prominent, and tended to disappear entirely in the more severely degraded samples (Figures 5–8 inclusive). In the early stages of degradation, expansion was still somewhat restricted but to a much lesser degree than in the original specimens (Figures 5 and 6). The fibers swelled much

more evenly, mushrooming was less prominent, and ballooning appeared to only a very limited extent. In later stages, evidence of restriction to expansion disappeared completely.

The most striking result of degradation was the appearance of one or two prominent spiral lines which appeared as spiral fissures as swelling proceeded to its limit (Figures 6, 7, and 8). In the early stages of degradation (as measured by breaking-strength loss) faint lines began to appear which in a few instances could definitely be identified as fissures. They usually appeared as two distinct lines running parallel and close to each other in the highly swollen fibers. Close examination suggested that these parallel lines were simply the edges of a single fissure. In some cases the parallel lines appeared to be two distinct fissures. As degradation proceeded, the fissures became deeper and increased in extent (Figures 7 and 8). In highly degraded fibers (49% loss in breaking strength) the fissures in some cases extended to the lumen, and the fiber on swelling opened into a ribbon similar to an untwisted drinking straw (Figures 8 and 10). The fissures extended the entire length of all the fiber sections. Also in the highly degraded specimens shallower secondary notches could be seen on the surface of the fibers (Figure 7). These notches are very probably the same as those observed by Great-house [9] in cotton fibers degraded by the fungus *M. verrucaria*.

Whenever both the spiral structure (winding) of the fiber and the spiral fissures could be observed (in the early stages of degradation) in a portion of a fiber, it was noted that the fissures and the spiral structure wound in opposite directions (*e.g.*, Z cuts appeared with S spirals). Then, too, it seemed that

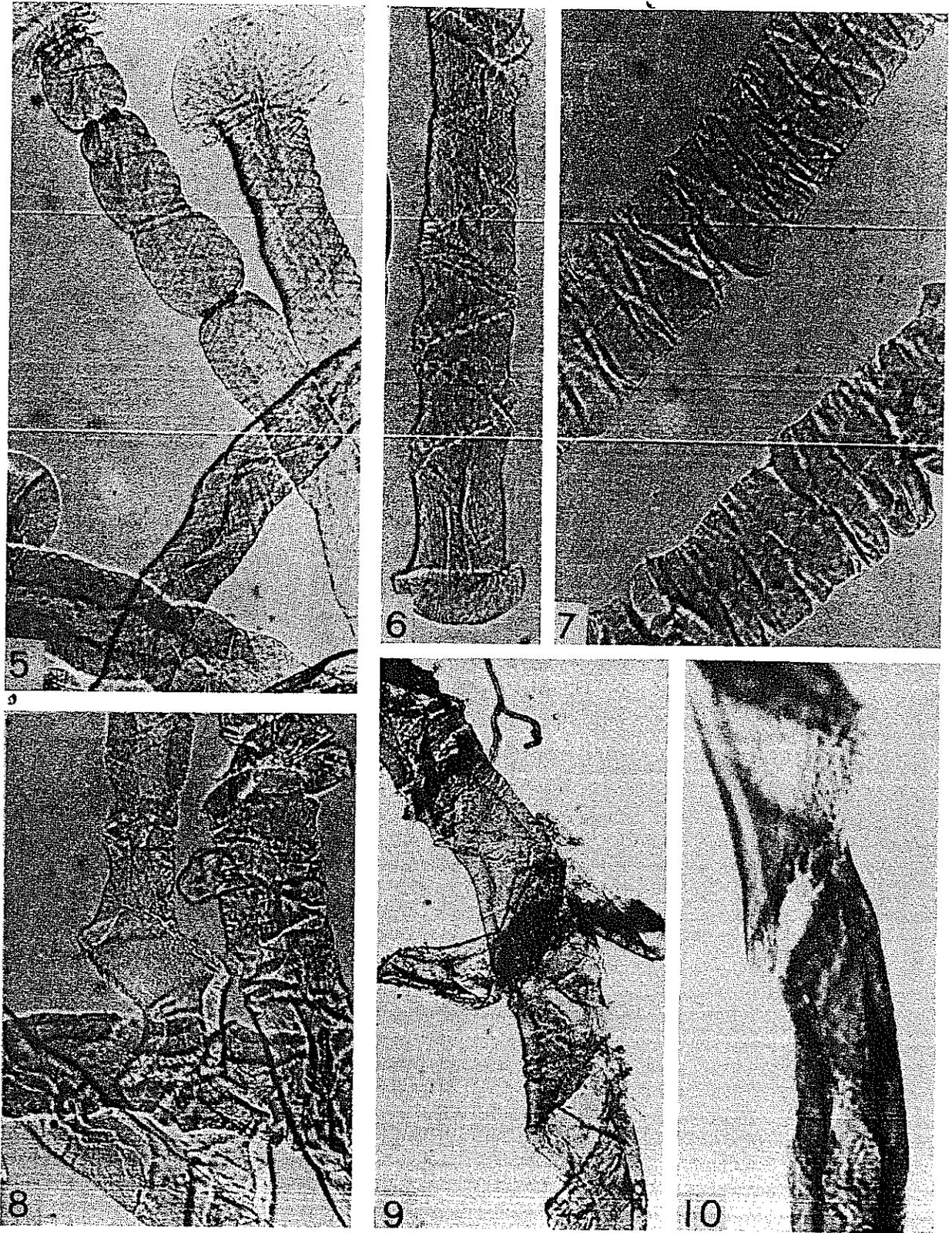


FIG. 5. Cotton fiber swollen in cuprien after being incubated for 61 days in salt solution at 40°C. FIG. 6. Fibers swollen in cuprien after being incubated for 5 days in enzyme solution at 40°C. FIGS. 7 and 8. Fibers swollen in cuprien after being incubated in enzyme solution for 27 days at 23°C. FIG. 9. Fiber from cotton duck degraded by *Curvularia lunata*, swollen in 8% NaOH. (All of the above at 238 × magnification.) FIG. 10. Fiber prepared by the Clegg procedure after incubation in enzyme solution for 27 days at 23°C. (Magnification, 538 ×.)

the spiral structure remained intact and passed over the fissures. It was also observed that the fissures would reverse their direction. Two types of reversals were found: either the fissure was continuous through the zone of reversal, or one fissure ended in the zone and another started alongside or shortly after the first. Anderson and Kerr [2] reported similar types of reversals of the first layer of the *secondary wall* in a histological study of normal cotton fiber. On any given portion of a fiber, one or two parallel fissures have been observed. More than two have not been noted.

All six samples of fungus-degraded duck which were examined were the same in appearance as the enzyme-degraded fibers. The above-noted visible evidence of damage varied in degree with the amount of growth in the neighborhood of the specimen. When growth was heavy, the specimens were similar in appearance to the fibers shown in Figures 7 and 8, except that the surface notches were deeper and more extensive and there was a greater tendency to untwist into a ribbon along the spiral fissures. Figure 9 shows the spiral fissures and untwisting in a fiber degraded by *Curvularia lunata*. The secondary notches were not brought out by swelling in sodium hydroxide and therefore do not appear in this photograph. Most of the mycelial growth was on the outside of the fiber. In only a relatively few specimens was growth observed in the lumen.

Bright [4] demonstrated cracking of the cotton fibers as a result of fungal degradation. He did not mention the spiral nature of these cracks, although a photograph in his paper strongly suggests that they have a spiral form. Clegg [5] in a later paper noted splitting along "quick" spirals in some cases of damage by *fungi*. The spiral cracks or fissures appear very prominently in degraded cotton swollen in cuprien, but are rather obscure (even when the fibers are highly degraded) in fibers swollen in the reagents used by the above authors (compare Figures 7 and 10). It is perhaps for this reason that the spiral nature of the cracks received only cursory attention by them.

Optical studies revealed several notable differences between the original and the enzyme-treated viscose rayon.

Examination of cross sections showed that the undegraded filaments were stained by Congo red only very lightly in a shell around the fiber. The de-

graded specimens were stained heavily in the outer portion and chiefly on the high points of the ridges. Cross sections (Figure 12) of degraded viscose stained by Morehead's technique to bring out the skin showed regions (usually on the ridges) which were unstained. These unstained portions in some instances extended completely through the skin to the core. No such discontinuities were found in the undegraded fibers (Figure 11). Electron photomicrographs (Figures 13 and 14) of surface replicas showed an even appearance for the original fiber and a diffuse blotchy appearance for the surface of the degraded fiber. These observations indicated that the enzyme had caused irregular erosion of the surface of the fiber.

Swelling in cuprien showed further differences between degraded and undegraded samples (Figures 15-19 inclusive).

When *undegraded viscose* was treated with cuprien the filaments swelled evenly, approaching a cylindrical form, and the ridges tended to disappear. As swelling proceeded, a distinct transparent cylindrical sheath appeared around the filaments (Figure 15). When pressure was exerted this sheath broke into many long, fine fibrils (Figures 16 and 17). Under relatively high pressure, the main body of the filament tore longitudinally into coarse pieces, with no clearly defined structure (Figure 17).

On swelling in cuprien, the *enzyme-treated viscose* exhibited some characteristic differences from the original filaments: The sheath became less distinct and tended to disappear as the time of incubation increased. On crushing, the fibrils produced from the sheath became fewer and shorter and finally disappeared with the disappearance of the sheath. Also, as swelling proceeded, longitudinal spindle-shaped cracks appeared spontaneously in the degraded fibers (Figure 18). These cracks varied in extent and degree. Some specimens showed a few shallow cracks with varying lengths. Others split so extensively and deeply that the filament was broken generally into anastomosing fibrils varying greatly in diameter. There appeared to be a general tendency of the degraded filaments to break up into a network of fibrils. This tendency was especially noticeable on application of pressure. Under very light pressure the filaments broke into the network system described above (Figure 19). Under increased pressure there was a further breakdown into finer fibrils, and the fibrils tore transversely.

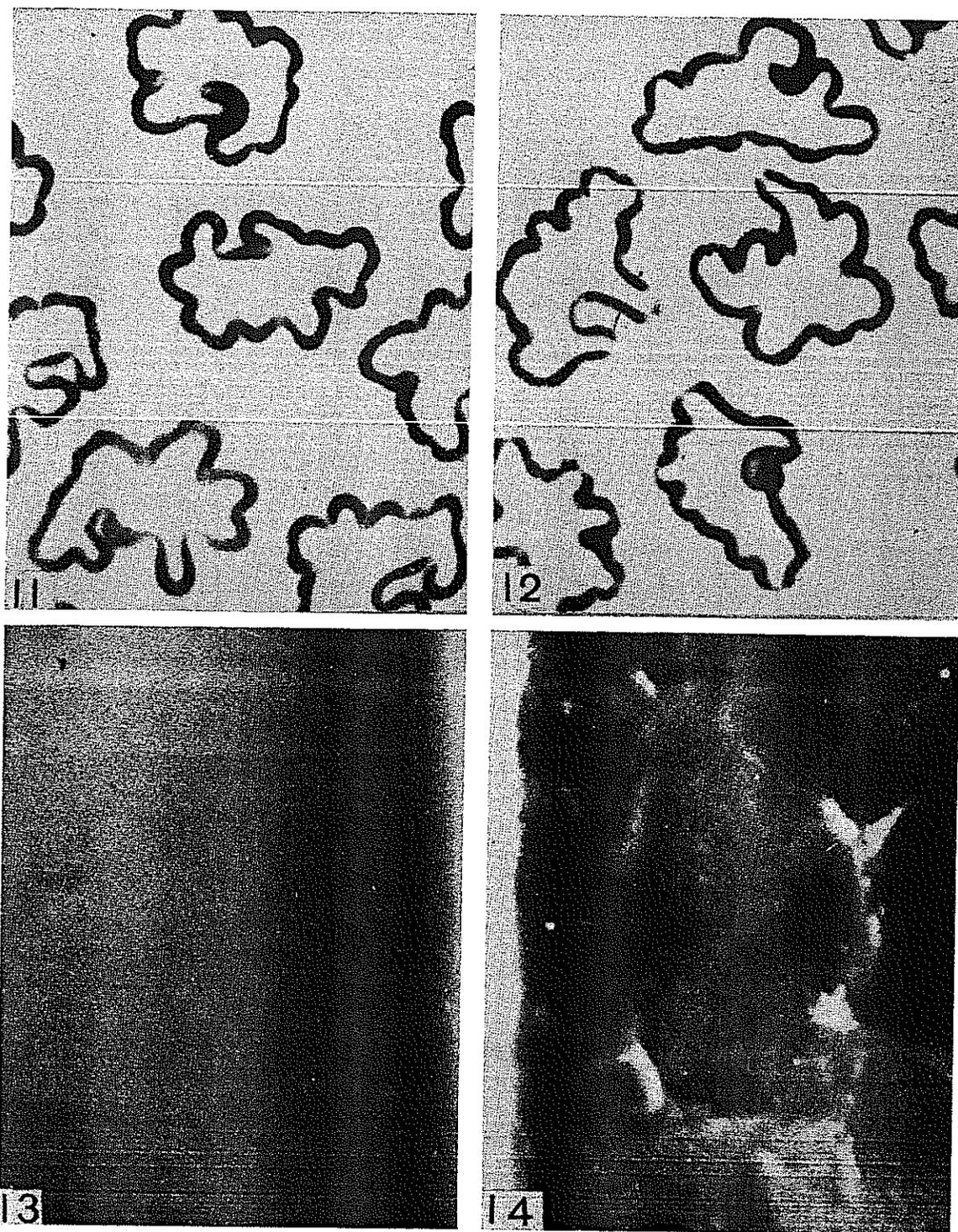


FIG. 11. Cross sections of viscose rayon filaments incubated for 5 days at 40°C in salt solution and stained to show skin. FIG. 12. Same as Fig. 11, but incubated for 5 days at 40°C in enzyme solution. (Magnification, 1500 ×.) FIG. 13. Electron photomicrograph of surface replica of a portion of a viscose rayon filament that was incubated for 5 days at 40°C in salt solution. FIG. 14. Same as Fig. 13, but incubated 5 days at 40°C in enzyme solution. (Magnification, 25,000 ×.)

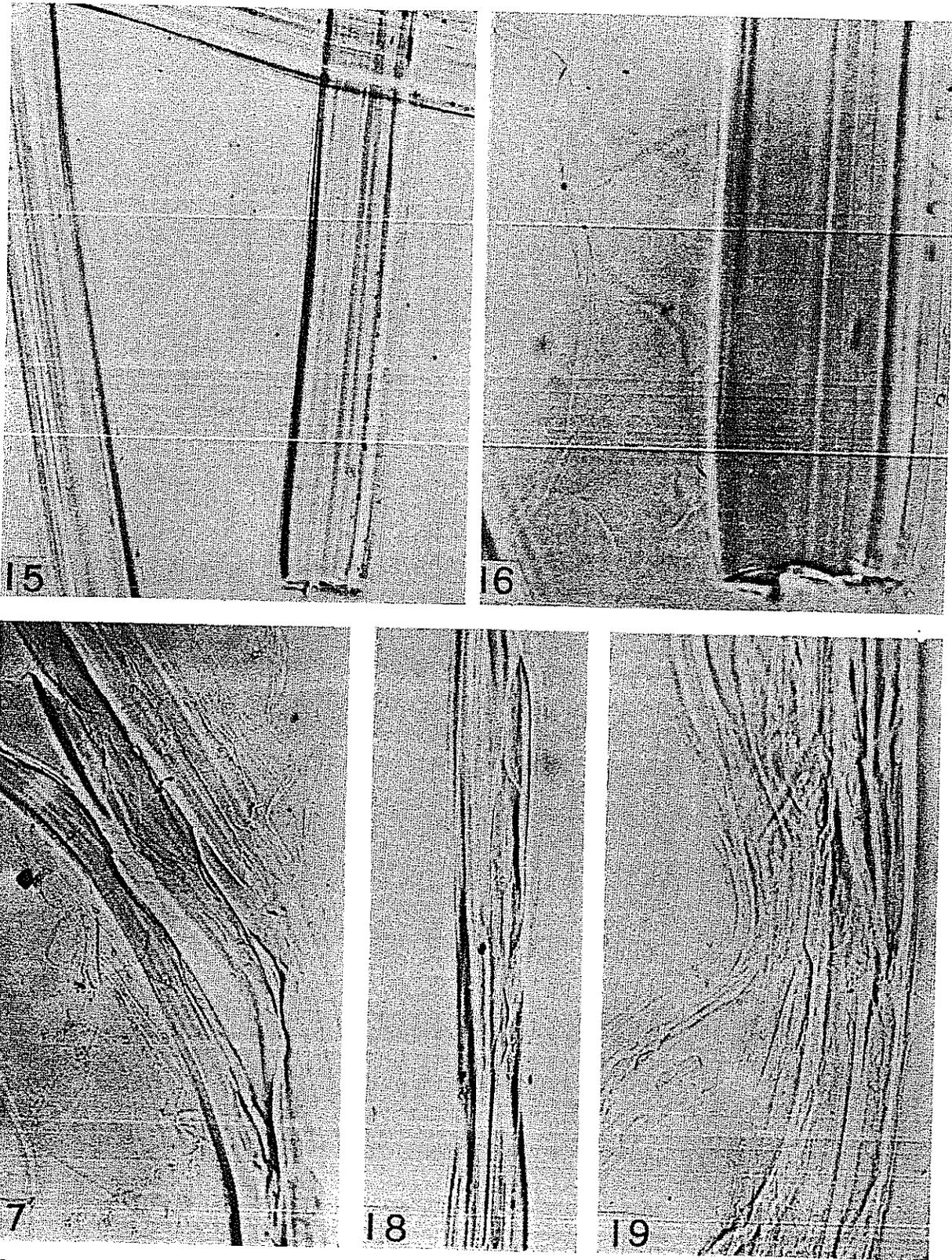


FIG. 15. Viscose rayon filaments incubated in salt solution for 39 days at 40°C and swollen in cuprien. (Magnification, 240 ×.) FIG. 16. Same as Fig. 15, but very light pressure was applied after swelling. (Magnification, 510 ×.) FIG. 17. Same as Fig. 15, but heavy pressure was applied after swelling. (Magnification, 240 ×.) FIG. 18. Filaments incubated in enzyme solution for 39 days at 40°C and swollen in cuprien. (Magnification, 240 ×.) The spindle-shaped cracks appeared spontaneously as the filaments swelled. FIG. 19. Same as Fig. 18, but light pressure was applied. (Magnification, 240 ×.)

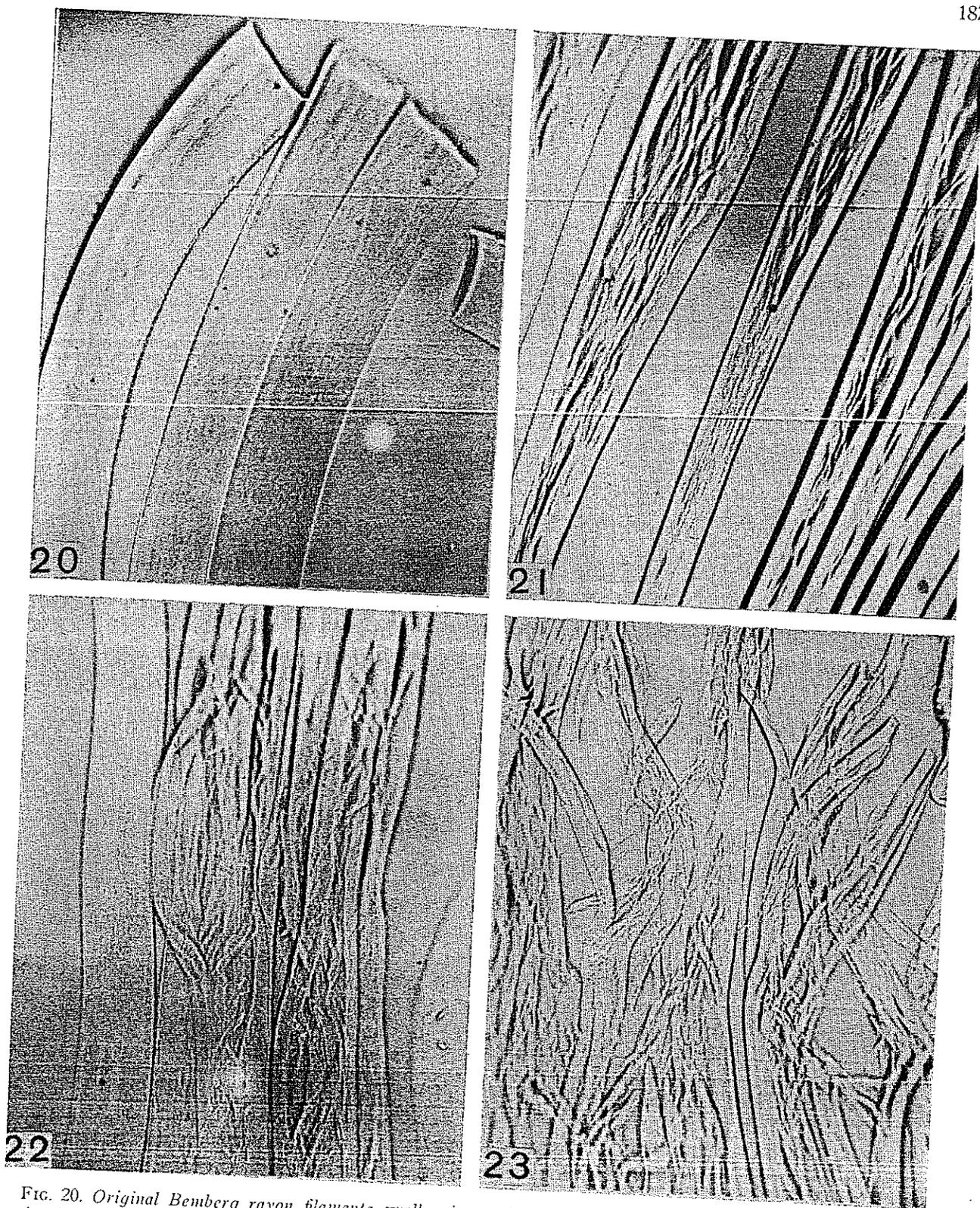


FIG. 20. Original Bemberg rayon filaments swollen in cuprien. FIG. 21. Original filaments swollen in cuprien and subjected to heavy pressure after swelling. FIG. 22. Filaments incubated 14 days in enzyme solution at 40°C and swollen in cuprien. Very light pressure was applied. FIG. 23. Same as Fig. 22, except that moderate pressure was applied. (All of the above at 240 \times magnification.)

The appearance of the Bemberg rayon specimens is shown in Figures 20-23 inclusive.

Both the degraded and undegraded Bemberg rayon filaments swelled in a similar manner in cuprien (Figure 20). They expanded evenly, with a strong tendency to bend. In neither sample were cracks of any kind visible. A difference between the degraded and undegraded specimens became apparent when the filaments were put under pressure. On application of *relatively high* pressure, the *undegraded* filaments broke into a network of fine anastomosing fibrils (Figure 21) qualitatively similar to the network formed in degraded viscose. The *degraded* Bemberg rayon filament also broke into fibrils, but only a *very light* pressure was required to produce extensive splitting (Figure 22). Under heavier pressure the fibrils tore transversely (Figure 23).

Discussion

The experimental evidence suggests that degradation of the cellulose fibers by the enzyme solution was influenced to a very large extent by the nature of the fiber itself. Because of the differences in the characteristics of the three fibers studied, it is perhaps preferable to discuss the degradation of each separately.

Cotton

The breaking strength and elongation at the breaking point of the degraded cotton fiber decreased rapidly with time of incubation in the early stages. The loss in tensile properties may be the result of a surface degradation and a consequent removal of successive layers from the surface of the fiber, an attack within the fiber mass, or a combination of both processes.

That the surface of the fiber was degraded is indicated by several considerations. This process might, of course, be expected to occur (on any cellulose fiber) since the enzyme is in contact with the surface and the enzyme does degrade cellulose. Experimentally, the deeper staining of the enzyme-treated fibers with Congo red indicates stripping of the fiber surface. The Congo red test is commonly used to show surface damage to cotton fibers. Further evidences of surface degradation are the changes in swelling characteristics observed under the microscope. The visible spiral structure and the restrictions to swelling, presumably due to this structure, disappeared.

Although the surface of the fiber was undoubtedly stripped in the degradative process, it is very unlikely that this factor alone can be responsible for the large and rapid loss in breaking strength (approximately 30% in 3 days) in the early stages. The weight loss of the fiber during this time was at most less than 0.5%. Even if the entire change in weight were a result of loss from the fiber surface, the change in cross-sectional area would still be too small to account for the load-elongation change. The large loss in breaking strength can be ascribed to surface degradation only by assuming the highly improbable possibility that a great part of the tensile strength resides in a thin surface layer of the fiber. Therefore, internal degradation is a more probable factor.

Measurements of crystalline diffraction and degree of polymerization showed no significant changes in these properties and therefore gave no indication of internal degradation. The observation of spiral fissures is the only positive experimental evidence for internal degradation of cotton fibers. It appears, therefore, that in the early stages at least the degradative action of the enzyme was restricted to the fiber surface and to spiral planes of susceptibility within the fiber mass. Furthermore, it is reasonable to believe that the rapid loss in tensile properties was primarily a result of degradation along these planes. This postulate leads to the expectation that the rate of loss would remain high until the depth of degradation along the susceptible planes approached the lumen (at which time the fiber loses its distorted cylindrical form and becomes, in effect, a flat ribbon); the rate would then abruptly decrease to a magnitude reflecting any continuing degradation which may occur in less susceptible regions of the fiber. This expectation is in agreement with the fact that, under the experimental conditions, no further loss in tensile properties had been observed after 3-5 days of incubation.

The fact that the enzyme lost activity during the incubation period (see Figure 1) necessitates some modification of the above reasoning. In general, the velocity of a reaction catalyzed by an enzyme is a direct function of the enzyme activity (up to a limiting value). Therefore, if the enzyme had not lost activity during the incubation period, it would be expected that the rate of loss of tensile properties would not fall to zero, but would have some relatively small value reflecting continuing degradation. That this is likely is indicated by the observation

that cotton fibers incubated for 27 days at 20°C (at which temperature the rate of loss of enzyme activity is appreciably less than at 40°C) lost approximately 50% of their original breaking strength (Figure 4), while at 40°C over the same period of time the fibers lost an estimated (interpolated) 35% of their strength. Nevertheless, considering that loss of activity is a continuous function of time (Figure 1) at a given temperature, it is hardly likely that activity loss of itself would result in an abrupt decrease to zero of the rate of loss of tensile properties. Such an abrupt change must then be related to some fiber property. Therefore, one is again led to a postulate of regions of high and low susceptibility in the cotton fiber.

The postulated relationship between rapid tensile-properties loss and degradation along spiral lines of susceptibility is in accord also with the findings of Stanier [20] in his work with *Cytophaga hutchinsonii*. The degradative attack by this organism is apparently restricted to the cotton fiber surface. Stanier noted (and his photographs show) that swollen, degraded fibers are covered with spiral furrows. (These furrows, incidentally, are somewhat similar in appearance to the notches (Figure 7) observed in fibers highly degraded by the enzyme solution.) He did not describe, nor do his photographs indicate, the occurrence of internal spiral fissures. Furthermore, Stanier found that "Cotton fibers, even when heavily invested with *C. hutchinsonii*, retain to a considerable degree their original structural and tensile properties." Thus, it appears that in this case the degradative attack results in no production of spiral fissures and in only relatively small changes in tensile properties—an inverse manifestation of the suggested relationship between the two occurrences. Moreover, it should be noted that even though the surface is highly degraded, little of the tensile properties are lost. This observation provides evidence for the assumption made earlier that surface degradation alone cannot account for the large and rapid loss of tensile properties.

The discussion thus far has been concerned primarily with the effect of a *cell-free extracellular cellulase solution* on cotton fibers. Practically, the effect of the cellulose-degrading *organisms* themselves are of primary importance. The findings of other workers studying the effect of fungi, combined with a few observations made by the present authors, suggest that degradation of cotton by at least some fungi

is fundamentally similar to degradation by the enzyme solution. Based on observations made by himself and many others, Greathouse [9] indicated that fungal degradation results in ". . . great loss in tensile strength with relatively small changes in chemical characteristics. . . ." Fleming and Thaysen [8], as well as many others, observed a decreased tendency of degraded fibers to balloon on swelling. Furthermore, as noted earlier, Clegg reported observing splitting along "quick" spirals in some cases of damage by fungi. The present authors have observed such splitting in *all* specimens degraded by six fungi. These effects caused by fungi are basically similar to the effects found to result from the action of the enzyme filtrates. Such a similarity is not surprising, since it is generally accepted that cellulolytic fungi elaborate an extracellular enzyme system which is primarily responsible for the degradation of cellulose.

It might be pointed out that because of the procedure for obtaining the enzyme filtrate used in these experiments, the filtrate undoubtedly is highly diluted and should therefore have a much lower cellulolytic activity than the enzyme system secreted directly on the fiber by the organisms. This condition perhaps accounts for the fact that while tensile-strength curves of fungal-degraded cotton given by Abrams [1] and Rogers, Wheeler, and Humfeld [16] show a definite decrease in loss rate well before this property is reduced to zero, it is neither as great in magnitude nor as abrupt as the change in rate of loss found for the enzyme-degraded cotton. The high enzyme concentration, then, would have the effect of increasing the rate of degradation in those regions of the fiber which are relatively resistant (as compared with the spiral planes of susceptibility) to enzyme action.

The spiral nature of the fissures and their reversals in direction naturally suggest a relationship to the spiral structure of the fiber. Anderson and Kerr [2] suggested that the spiral structures of the first few outer layers of the cotton fiber do not successively wind in the same direction, but that a template is soon established and thereafter successive internal layers all follow the same direction. Hock, Ramsey, and Harris [11] believe that the orientation of the fibrils immediately below the winding and of all subsequent layers are always in a direction opposite to that of the winding. Since the observed fissures in the degraded fibers have been

found to spiral in a direction opposite to that of the outer winding, it appears, in view of the findings of Hock *et al.*, that the fissures have the same direction of spiral as the internal layers of the fiber.

Viscose Rayon

Degradation of viscose rayon by the enzyme filtrate resulted in early rapid loss in tensile properties, an appreciable loss in weight, and extensive surface degradation. In this case surface degradation could conceivably account for an appreciable part of the tensile loss.

The relative ease with which the swollen, degraded filament was split into anastomosing fibrils suggests severe degradation between these fibrils. It is, therefore, not unreasonable to believe that interfibrillar degradation contributed in a large, if not major, measure to tensile-properties loss.

The negative results of the degree of polymerization and crystallinity measurements indicate that very little or no intrafibrillar degradation occurred.

The rather sharp decrease in the rate of tensile-properties loss again suggests that the degradative process resulting in such loss is a combination of two processes occurring at greatly different rates. The rapid rate process is perhaps degradation of the filament surface and of the interfibrillar cellulose. The slow rate process could be continuing degradation of the fibril surfaces.

Bemberg Rayon

Bemberg rayon incubated with the enzyme solution changed rapidly and radically in all measured properties within 3 days. Such changes clearly indicate that the degradative process in this material occurred rapidly throughout the entire filament mass. The ease with which the swollen, degraded filament was broken into fibrils suggests that the interfibrillar regions were degraded to a somewhat greater degree than were the intrafibrillar regions.

Of interest is the observed rapid abnormal loss of activity of the enzyme in contact with Bemberg rayon in contrast to the normal loss in the presence of cotton and viscose rayon. A few speculative thoughts on this subject are perhaps permissible. Such a loss may be a result of either inactivation or removal of the enzyme from solution by adsorption on the fiber surface. The former alternative does not appear very likely since no measurable abnormal activity

loss was found in the presence of cotton and viscose rayon. The degree of adsorption would be a function of the surface area available to the enzyme. If adsorption is to account for the activity loss, then the results suggest that Bemberg rayon has a very much greater available surface area than does cotton or viscose rayon. Though both of the latter fibers have a fairly large external surface, it is apparently insufficient to absorb any measurable amount of enzyme. The large Bemberg surface area may be either external (the filament surface) or internal. The latter possibility is in accord with the observed very rapid change of tensile and other properties during the degradation process.

A concept of regions of high susceptibility was introduced, with no attempt made to define the nature of such regions. Some clarification of this concept is perhaps desirable. Regions of high susceptibility are identical with those regions which, on exposure of the fiber to the enzyme solution, showed a tendency to become weaker in the swollen state—*i.e.*, the observed spiral-fissure planes in cotton and the interfibrillar split planes in viscose and Bemberg rayons. Thus, the locations of these regions are defined experimentally; their nature, however, is not.

While the available factual information is insufficient at present to enable one to offer an incontestable explanation of such regions, two possibilities appear plausible:

The rate of degradation of a cellulose mass depends upon the number of active bonds (1-4- β -glucosidic linkages) accessible to the enzyme. Because of the large size of the enzyme molecule, it can be expected that not all of the internal bonds would be accessible, and that the number and location of the accessible bonds would be conditioned by the dimensions and distribution of the voids in the cellulose mass. The regions of high susceptibility then would be those regions containing spaces of such dimensions as to allow the enzyme to penetrate readily.

Localized variations in density related to the relative amounts and distribution of amorphous and crystalline cellulose may reasonably be expected to play an important role in determining the paths of rapid degradation. Many workers [18] have demonstrated that amorphous cellulose is degraded much more rapidly by enzyme than is an equal mass of crystalline cellulose. Furthermore, low-density

amorphous regions contain fewer linkages per unit volume than do the high-density crystalline regions. It follows, then, that the enzyme molecules can degrade the cellulose in a given volume more rapidly in amorphous regions than in crystalline regions. Therefore, if the distribution were such as to form lines of successive amorphous regions, paths of low density (and therefore high susceptibility) would result.

Accessibility and density have been presented separately as possible explanations for the observed regions of high susceptibility. They are not, however, mutually exclusive. It is conceivable that both are factors and that the relative importance of each is dependent upon the characteristics of the cellulose mass under consideration.

Summary

The early stage of degradation of cotton fiber by a cell-free enzyme solution prepared from a cellulolytic fungus was strongly characterized by a rapid loss of tensile strength. Neither at this stage nor at any later stages were there detectable changes in degree of polymerization or crystallinity. Microscopic observation of the swollen fibers revealed a gradual disappearance of the normal outer spiral winding and the appearance of spiral fissures which became more extensive and deeper with increasing incubation time.

The initial rapid loss of tensile properties is attributed to rapid degradation along spiral planes and a further slow loss to continuing attack on the exposed surfaces, resulting in general erosion and localized notching. Fungal degradation appears to occur in essentially the same manner as degradation by the enzyme solution.

Changes in the measured properties of enzyme-degraded viscose rayon were qualitatively similar to the changes of degraded cotton. Microscopic evidence suggested that rapid loss of tensile properties was caused by surface and interfibrillar degradation by the enzyme. Further slow loss in tensile properties may be ascribed to continued attack of the accessible external and interfibrillar surfaces.

Bemberg rayon was very rapidly and extensively degraded by the enzyme solution. Within 3 days the tensile properties dropped to negligible values, degree of polymerization decreased to approximately 30% of that of the original material, and the degree of crystallinity increased. During the course of deg-

radation the rate of weight loss was very high initially and then decreased rapidly when 20%–25% of the cellulose had been solubilized. The relative ease with which the swollen, degraded filaments were broken into fibrils indicated that the degree of degradation was greater between than within the fibrils.

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