PHYSICAL AND CHEMICAL CHANGES PRODUCED IN BLEACHED COTTON DUCK BY

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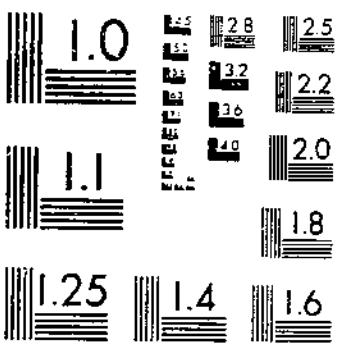

MICROCOPY RESOLUTION TEST CHART
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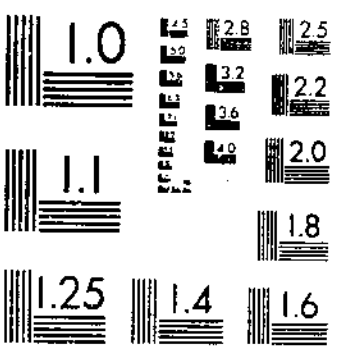

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Physical and Chemical Changes Produced in Bleached Cotton Duck by Chaetomium globosum and Spirochaeta cytophaga[1]

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CONTENTS

INTRODUCTION

The yearly loss known to be due to the deterioration of fabrics by micro-organisms is considerable, and a great deal of damage of textiles now ascribed to other causes results from the unrecognized action of fungi and bacteria. Under the conditions of temperature and moisture usually encountered, fungi are mainly responsible for cellulose attack. As the moisture content of the textiles increases, bacteria become relatively more important than fungi. These organisms usually need only favorable conditions of humidity and temperature to cause damage. The conditions especially favorable to their activity are found in seacoast communities and during the summer in many Southern and Midwestern States.

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The micro-organisms that affect cotton may be divided roughly into two groups: (1) Those that only discolor the fibers, and (2) those that actively attack the cellulose and hence cause a loss in breaking strength. Some micro-organisms belong to both groups since they discolor as well as weaken the fiber.

Cotton fibers at the time of boll opening contain constituents other than cellulose which make the fiber more subject to damage by micro-organisms than is cellulose alone. These substances are both organic and inorganic in nature. The organic constituents furnish food to these heterotrophic micro-organisms, while the inorganic supply the mineral elements essential for their growth. The organic fraction, although present in comparatively small amounts, enables other organisms, as well as those that destroy the cellulose, to grow and discolor and perhaps tender the fiber.

After the boll opens, the cotton becomes contaminated with micro-organisms and accumulates more or less dust and dirt. These furnish additional material to support growth of micro-organisms. Thus, at favorable temperatures and humidities, cotton may deteriorate considerably before picking. And after picking, ginning, and baling, cotton often is stored under conditions that favor further deterioration.

Manufacturing processes are normally performed under conditions that increase this liability to damage. Warp yarns are sized with constituents that accelerate growth. Although bleaching and other processes remove many of the impurities in raw cotton, so that bleached fabric is less subject to damage than unbleached material, other treatments such as dyeing and finishing may again furnish substances that stimulate growth of micro-organisms.

Then, during wear, the fabric becomes soiled, and this accumulation of dirt and grease, however small, may accelerate their growth. It is evident, therefore, that the cotton fiber is subject to damage by micro-organisms from the time the cotton boll opens until the fiber is finally decomposed.

In a preliminary study, one of the authors developed a method (*35*)[3] for testing the effectiveness of mildewproofing agents on cotton fabrics in which the fungus, *Chaetomium globosum*, Kunze is used as the test organism. This particular fungus was selected because it was found on nearly all outdoor fabrics used for awnings, tarpaulins, shock covers, etc. Laboratory tests on the cultures isolated from these materials indicated that this chaetomium was one of the most important organisms responsible for the loss of breaking strength of fabrics. This test method has been adopted by several research laboratories of industrial concerns, and is used to test mildewproofed materials now purchased under certain Government specifications.

The method also lends itself to the study of the deterioration of cotton and other cellulosic materials. During incubation favorable conditions approaching the optimum are maintained. Deterioration is accelerated so that as much damage occurs in 2 weeks as would require months in the field. The present investigation was undertaken to secure information needed concerning the various physical and chemical changes which undoubtedly accompanied the loss of breaking strength produced by the chaetomium.

[3] Italic numbers in parentheses refer to Literature Cited, p. 33.

During a study of soil organisms, a bacterium was isolated that proved to be a very active cellulose decomposer. This bacterium was identified as *Spirochaeta cytophaga*, Hutchinson and Clayton. It was found that *S. cytophaga* grew well under the conditions maintained for the chaetomium test.

Since the manner of growth and the actions of fungi and bacteria are quite different, a comparison of the activity of the chaetomium and the spirochaete was made in the present investigation. The action of these organisms on bleached cotton duck was determined by measuring changes in breaking strength, weight per square yard, thickness, staple length, fluidity in cuprammonium hydroxide, copper number, methylene blue absorption, moisture, and ash. The rate of evolution of carbon dioxide was chosen as an indication of the rate of growth of the organisms on the fabric. The changes produced by the organisms as indicated by the results of the analyses were compared and were analyzed statistically to discover similarities and differences among the results and between the actions of the two organisms.

REVIEW OF LITERATURE

Most of the published reports on the microbiology of cotton deal with the identification of micro-organisms found on cotton fibers and fabrics. Almost no quantitative data on their effect on cotton textiles are available. Although a great number of species of organisms occur on cotton, many of them do not produce mildew. The investigations that report the presence of micro-organisms on cotton are listed below in chronological order.

In 1880 Davis, Dreyfus, and Holland (*11*) stated that the spores of the fungi causing mildew are constantly present and that cotton fabrics exposed to warmth and moisture are likely to mildew. They identified several species of fungi taken from mildewed cotton materials.

Holle (*19*) investigated fungoid growths on cotton fibers and concluded that the cotton hair was infected before the cotton was fully ripe. He stated that the hyphae of fungi penetrated through the outer wall of the fiber into the lumen.

A fungus that produced a pink color in cotton was isolated but not identified by Trotman (*36*).

Osborn (*22*) investigated some micro-organisms infecting various types of cloth and was able to isolate species of *Penicillium*, *Mucor*, *Fusarium*, *Aspergillus*, *Stysanus*, *Chaetomium*, and several unidentified bacteria and phycomycetes.

Broughton-Alcock (*6*) stated that fungi of the genus *Macrosporium* and of *Stemphylium* were principally responsible for the microbiological destruction of cotton and linen canvas.

Sidebotham (*30*) found that *Botrytis* caused discoloring and tendering of dyed cotton cloth. Growth was rapid at 90° to 100° F.

Aspergillus niger and a species of *Penicillium* were isolated by Armstead and Harland (*2*) from cotton fabric shipped from India to England. They found that at humidities ranging from 90 to 100 percent *A. niger* grew on the unsized fabric and the *Penicillium* species on the sized material.

Bright, Morris, and Summers (*5*) stated that the fungi most commonly found on cotton are species of *Aspergillus*, *Rhizopus*, and *Penicillium*, and that they may occur on the material at any stage of manufacture from the raw cotton to the finished fabric. These investigators discredit the opinion that acids produced by the micro-organisms cause tendering of the fabrics.

In 1924 Thaysen (*34*) published a general discussion concerning the growth of different types of micro-organisms and of their effects on cotton fibers and fabrics. He stated that bleached cotton is more resistant to microbiological attack than raw cotton, and concluded that this difference probably is due to a difference in the protein content of the two materials.

Shapovalov (*29*) found that *Aspergillus niger* and *Rhizopus nigricans* frequently caused a rotting of young cotton bolls. These organisms were able to attack only bolls that previously had been damaged mechanically or by insects.

Smith (*31*) stated that the following species of *Aspergillus* infect cotton: *A. flavus*, *A. fumigatus*, *A. niger*, *A. repens*, *A. rubber*, *A. sydowi*, *A. terreus*, *A. versicolor*, and *A. wentii*. He also reported (*32*) that the various species of fungi which attack cotton differ considerably in their behavior. Some grow at low temperatures and high humidities, and others at fairly high temperatures and low humidities. Certain species attack the cellulose; whereas others only discolor the fiber. Some grow best on the fiber, and a number utilize principally the sizing materials in the fabrics.

Galloway (*13*) listed species of the following genera as among the fungi most prevalent on cotton goods: *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Rhizopus*, *Cladosporium*, *Stemphylium*, *Macrosporium*, *Botrytis*, *Chaetomium*, *Helminthosporium*, *Dematium*, *Trichoderma*, *Monilia*, and *Actinomyces*. Of these, *Aspergillus* and *Penicillium* were found most frequently. He stated that some of these organisms were able to produce an appreciable loss of strength, but that a concentration of 25 percent or more of carbon dioxide in the atmosphere inhibited their growth. Later he (*14*) studied the occurrence of "diamond spot" mildew and found that it was caused by the growth of the fungi along the warp and filling directions of the fabric from the point at which growth originated. A number of fungi such as *A. niger*, *A. terreus*, and species of the genera *Fusarium*, *Cladosporium*, and *Helminthosporium* were found to produce "diamond spot."

A bacterium that caused yellow stains on skeins of cotton kept in a moist atmosphere, was isolated by Brussoff (*7*). He stated that a spore-forming, gelatin liquifying, rod-forming bacterium could always be isolated from these stains.

Heyes and Holden (*18*) isolated two species of *Penicillium* from mildewed cotton goods, one of which resembled *P. purpurogenum*. They found that these two species and *P. pinophilum* were able to deteriorate cotton yarn.

Galloway (*15*) studied the length of time required at 25° C. at different humidities for the germination of the spores of a number of species of fungi isolated from cotton goods. The minimum relative humidity that permitted growth varied from 75 to 95 percent, depending upon the species of the fungus. He mentions

Rhizopus, *Trichoderma*, *Stachybotris*, *Thielaviopsis*, *Cladosporium*, *Stemphylium* and *Acrothecium* as able to grow at these humidities. Later he (*16*) observed that the principal fungoid damage to cotton occurs when cotton is not excessively moist.

Another type of cotton-fiber deterioration is described by Gulati (*17*). The micro-organisms enter the lumen of the fiber wherever it is mechanically damaged and burst the fiber by means of pressure built up within it. Some 17 species of fungi and 3 types of bacteria were isolated from cotton by this investigator.

Prindle (*24*, *25*) found that the fungi and bacteria on raw cotton were largely soil organisms. The fungi belonged to the genera *Hormodendrum*, *Fusarium*, *Alternaria*, with *Aspergillus* and *Penicillium* occurring in smaller numbers. Later he (*27*) studied the growth of micro-organisms on cotton fiber at different humidities and found that only *Penicillia* and *Aspergilli* grew over the range of humidities from 82 to 95 percent. A large number of actinomycetes were observed on cotton incubated at 95 percent relative humidity and 25° C.

The above-mentioned investigations were concerned chiefly with the identification of various micro-organisms found on cotton materials. Those listed below report methods for estimating the extent of mildew activity.

Veitch and Levine (*39*) tested mildew resistance by incubating disks of fabric on agar for periods of 7 to 10 days at room temperature. Then they examined the fabric and noted the color and the character of growth, and measured the size of the colonies. Fleming and Thaysen (*13*) counted the number of damaged and undamaged cotton fibers under the microscope after they were treated with caustic soda and carbon disulfide, and Bright (*4*) counted them after staining with Congo Red.

Prindle (*26*) noted the strength of cotton yarn treated with a number of molds isolated from raw cotton by breaking the yarn between his fingers. He first prepared a cotton-extract broth. Then he suspended small skeins of the cotton yarn in the broth and inoculated the skeins with a number of species of *Aspergillus*, *Hormodendrum*, *Oospora*, *Penicillium*, and *Mucor*. All of the micro-organisms grew well in the medium. Only one, a species of *Hormodendrum*, caused a complete loss of strength of the yarns, while two cultures of green *Penicillia* produced a partial loss. He also tested some bacterial cultures, but found none that reduced the strength of the yarn.

Searle (*28*) determined the wet breaking strength of mildewed fabrics to evaluate damage. He developed a method in which 15- by 1½-inch strips of cotton fabric were wound on filter candles which previously had been coated with a soil suspension. These strips then were incubated for 3 or 6 weeks by placing each candle in a test tube containing a small quantity of water. A loss of 55 to 93 percent in strength occurred during 6 weeks' incubation. Searle's method failed to give close agreement between loss of breaking strength of replicates under apparently identical conditions.

Thom, Humfeld, and Holman (*35*) determined the dry breaking strength of mildewed duck. They abandoned mixed cultures because of the difficulty in obtaining comparable results and measured the activity of a single organism under controlled conditions. By test-

ing a number of commercial mildewproofing agents they found that some prevented the growth of their test organism, *Chaetomium globosum*, but that others were ineffective in this respect.

These studies show that, in general, only two types of methods have been used to measure the extent of mildew activity. These are microscopic examination and determination of breaking strength. A number of additional methods, both physical and chemical, were used in the present investigation of microbiological deterioration. Carbon dioxide evolution, which often is employed for measuring the rate of growth of micro-organisms, was adapted to the study of fabric deterioration. Also a differential staining method was developed for showing the presence of hyphae in cotton fibers.

EXPERIMENTAL PROCEDURE

Preparation and Sampling of Fabric

A bleached 14-ounce cotton duck with two-ply warp and single filling yarns was used for this investigation. It was washed and desized before it was given any bacteriological treatment. For the washing procedure a temperature of from 60° to 65°C. was maintained and a neutral soap solution was used. After washing, the fabric was thoroughly rinsed in warm distilled water to remove all traces of soap. It was then given a treatment with a starch and protein-solubilizing enzyme preparation (*7*) and finally was thoroughly rinsed.

The cotton duck, after inoculation with *Chaetomium globosum* and with *Spirochaeta cytophaga* was incubated for different lengths of time. For each period with each micro-organism, a 24-inch square of duck was sampled as shown in figure 1. The 6-inch squares designated as sw and sf were used for sample warp and filling breaking-

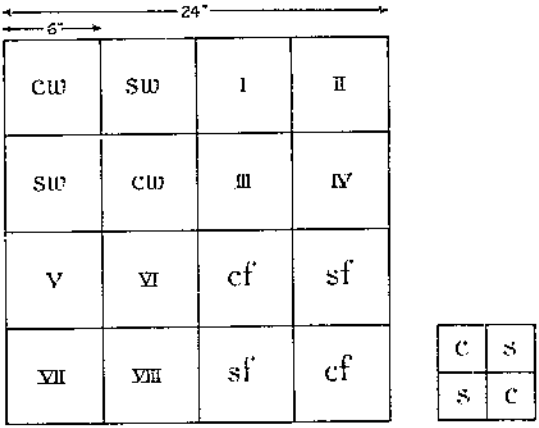


FIGURE 1.—Plan of lay-out for sampling the duck.

strength strips, respectively, and those marked cw and cf for control warp and filling strips. Controls from the immediate vicinity of the sample and of the same warp and filling were analyzed in order to compensate for variability of the fabric.

The squares marked I and II were used for determinations of weight, then thickness, moisture, and finally ash; III and IV for copper numbers; V for methylene blue absorptions; and VI for fluidity measurements. Pieces VII and VIII were used for staple length and for any experiments other than breaking strengths which needed to be repeated. Each of the eight squares designated by roman numerals were divided into control and sample pieces as shown in the small square in figure 1. For each replicate, material was taken from both control squares of the 6-inch piece and also from both corresponding sample sections.

Strips of fabric 6 by 1½ inches were taken for the carbon dioxide determinations. In this case a random method of sampling was used. These strips are not shown in figure 1 since the measurements were made after all the other tests had been completed. The close relationship that was found to exist between breaking strength and rate of growth suggested that pertinent information might be obtained by studying the rate of evolution of carbon dioxide. This measurement frequently has been used to investigate growth relationship.

Bacteriological Procedure

In order to sterilize the cotton duck, the strips were first placed in 16-ounce screw-cap bottles and the squares in Petri dishes 115 mm. in diameter and 15 mm. in depth. The strips and squares were wet out by filling the bottles and dishes with water. The water then was drained off and the bottles and dishes were sterilized in an autoclave for 1 hour at 15 pounds pressure.

A culture medium of the following composition was used: $NaNO_3$, 3 gm.; K_2HPO_4, 1 gm.; $MgSO_4$, 0.25 gm.; KCl, 0.25 gm.; agar, 10 gm.; and water, 1 l. The hydrogen-ion concentration of the medium was approximately pH 6.8.

The medium was melted and 50 ml. of it was poured into each of several 16-ounce screw-cap bottles. It was then sterilized in an autoclave at 15 pounds pressure for 20 minutes. The bottles were placed on their sides in a horizontal position and the agar was allowed to harden. This gave a layer of agar in each bottle about 5 to 6 mm. deep. After the medium had cooled, a sterile strip of fabric was placed on the surface of the agar in each bottle.

By adding about 30 ml. of medium that had been sterilized in bottles to each 115-mm. Petri dish, approximately the same depth of agar was obtained as in the bottles. A sterile square of fabric was placed on the agar in each dish. The strips and squares were transferred with a pair of long forceps which were flamed to render them sterile. The fabric samples were incubated for 2 or 3 days to make certain that no contamination occurred during transfer.

Stock cultures of *Ch. globosum* and *S. cytophaga* were kept in test tubes on filter paper that had been placed on the surface of the agar medium described above. Transfers were made to sterile filter paper on the mineral medium 10 days to 2 weeks before the culture was required for the inoculation of the fabric.

The inoculum was made for both the fungus and the bacterium by scraping off the growth from the surface of the filter paper and suspending it in bottles of sterile tap water. The resultant suspensions

were examined under the microscope to be certain that they were sufficiently concentrated to insure a thorough inoculation. The presence of a number of spores in each field examined was taken as an indication that the suspension of the fungus was satisfactory. In order to determine whether enough bacteria were present, a drop of the suspension of the bacterium was placed on a slide, stained with carbol fuchsin, and examined under the microscope. The presence of numerous cells in the stained preparation left no doubt as to the abundance of the bacteria.

One series of strips and squares then was inoculated with a culture of *Ch. globosum*. The strain used was one isolated in this laboratory from mildewed canvas and identified from Chivers' monograph (*10*). The culture subsequently was sent to Chivers who confirmed the identification. It had been employed repeatedly for testing the effectiveness of chemical mildewproofing agents, and was known to be one of the most active cellulose decomposers available.

A similar series of strips and squares was inoculated with *S. cytophaga*, a bacterium described by Hutchinson and Clayton (*21*). The strain used was isolated in this laboratory some time previously. The organism was undoubtedly the same as that isolated and described by Hutchinson and Clayton. In young cultures there was a preponderance of the threadlike, fairly long, thin, filamentous, and frequently curved cells. In older cultures, the "coccus" form predominated. Since these forms could not be separated, it was concluded that the strain was a pure culture. Its appearance agreed in all particulars with the description and illustrations of Hutchinson and Clayton, and therefore there seemed to be no reasonable doubt regarding the correctness of the identification. Cellulose was essential for its growth, and no growth was obtained by culturing on any other medium. It utilized filter paper, cotton fabric, and cellulose suspended in agar.

Inoculation was accomplished by transferring 2 ml. of the inoculum with a sterile pipette to the surface of each strip and square of fabric. The suspension was distributed over the entire surface of each fabric sample by placing the tip of the pipette at one corner of the piece of material and allowing it to slide back and forth over the surface of the fabric and at the same time letting the inoculum run out of the pipette.

A sufficient number of strips and squares was inoculated at one time to provide all that were needed for the physical and chemical analyses. The strips and squares were incubated for different periods at room temperature (about 28° C.). For the chaetomium the lengths of time of incubation were 1, 2, 3, 6, 9, 12, and 15 days, and for the spirochaete 2, 4, 6, 9, 12, 15, and 18. At the end of each incubation interval the required number of samples were removed from the incubator and each strip and square was washed free of surface growth, then air dried. Since the squares were to be used for chemical tests, they then were steeped and washed in 10 changes of warm, distilled water. The control squares were treated likewise.

Test Methods

The samples for the physical measurements were conditioned at least 4 hours and tested in a laboratory maintained at 70° F. and 65

percent relative humidity. The breaking-strength measurements were made upon strips 6 inches long and 1 inch wide, using the motor-driven Scott tester (*38*). For the weight determination, 2-inch squares were stamped out with a die and weighed on a torsion balance reading directly in ounces per square yard. Thickness was measured on the weight samples with a micrometer gage which exerted a constant pressure on a given area of the fabric and which was graduated to read to 0.001 inch.

The fiber staple lengths were determined with the Suter-Webb sorter (*1*). Warp and filling yarns were raveled from the duck and each type of yarn was untwisted carefully to separate its component fibers. After the cut fibers at the end of the yarns were discarded, the remaining fibers were arranged as nearly parallel as possible, and then each sample was sorted with the Suter-Webb instrument, measured, and finally weighed under standard conditions.

For the fluidity tests, 0.5-percent dispersions of cotton in cuprammonium hydroxide were used. The fluidities of these dispersions were measured with capillary-tube viscometers at 25° C. (*37, p. 53*). Copper numbers were determined on 1.5-gm. samples of finely divided fabric which were treated with Braidy's solution and heated for 3 hours in an oil bath thermostatically controlled at 100° (*37, p. 56*). Methylene blue absorption measurements were made on 1-gm. samples with a buffered methylene blue solution of pH 7 (*37, p. 29*). For the moisture determinations, 5-gm. samples of the conditioned material were dried at 105° to constant weight in special bottles designed by Barritt and King (*3*). The percentage of ash was determined by igniting the moisture samples to constant weight in a muffle furnace.

Carbon dioxide evolution was measured by passing air over a 6- by 1½-inch inoculated strip of duck that had been placed on the agar medium in a 16-ounce screw-cap bottle, and then by absorbing the evolved CO_2 in a solution of KOH. The milliequivalents of CO_2 given off each day by 1 gm. of fabric (on the basis of dry weight) was calculated. The strips inoculated with the chaetomium were tested daily for 15 days, and those with the spirochaete daily for 18 days.

The CO_2 method described by Humfeld (*20*) was modified in such a way as to sterilize the air in order to prevent contamination of the culture, to remove CO_2 from the air, and to refine the analysis of the solutions of KOH. The last change was necessary since only a relatively small amount of CO_2 was evolved by the action of the microorganisms on the duck.

In this modified method the air was bubbled first through concentrated H_2SO_4 in order to sterilize it, then through N KOH to remove CO_2, through a sterile empty bottle that served as a trap, and through a bottle of sterile water to humidify it. This sterile, CO_2-free, humidified air was then passed over the surface of the freshly inoculated fabric strip. It was introduced at the rear of the bottle containing the strip and removed at the front, after which it was passed through three bottles which contained 400 ml. of standardized N/10 KOH. Usually all of the CO_2 was absorbed by the KOH contained in the first bottle, but since the KOH solutions were very dilute, a small amount of CO_2 occasionally was carried over into the solution in the second bottle. The solution in the third bottle served as a control. For the chaetomium cultures the air movement was

provided by water aspirators controlled with needle valves, and for the spirochaete cultures, by a vacuum pump. It was possible to obtain a more uniform flow of air with the vacuum pump than with the water aspirators.

The CO_2 absorbed by the KOH solutions was precipitated as $BaCO_3$ by adding 10 ml. of a 2N $BaCl_2$ solution to each 400 ml. of KOH solution. Each solution was diluted to exactly 500 ml. with CO_2-free water, shaken, and allowed to stand until all the $BaCO_3$ had settled and the supernatant liquid was clear. Two aliquot samples, 100 ml. each, were removed by a pipette from each 500 ml. of solution, and each aliquot was placed in a 300 ml. Erlenmeyer flask containing 40 ml. of standardized 0.02 N HCl, sufficient to make the solution definitely acid. These solutions were then titrated with the N/100 KOH, using methyl red as the indicator. This back acid-alkali titration method was used since the excess acid present prevented any absorption of CO_2 from the atmosphere and subsequent precipitation of $BaCO_3$ during titration.

A method was developed for staining the fibers of the fabric incubated with *Ch. globosum* so that the hyphae of the fungus would be visible. It was difficult to demonstrate the presence of the fungus on the fibers because the very fine hyphae were colorless and therefore could not be seen unless stained. When the usual methods of staining were tried, the cotton fiber as well as the hyphae absorbed the stain.

In this new method a small sample of the fibers is immersed in a 0.1-percent basic fuchsin solution in 95-percent ethyl alcohol. After a few minutes, the fibers are removed and placed on a piece of filter paper which takes up the excess stain. The fibers next are immersed in a 1-percent aqueous solution of phenol. The length of time they remain in the phenol is unimportant. The sample then is placed on a slide in a drop of the phenol solution, covered with a cover glass, and examined. The usual mounting media either decolorize the hyphae or cause a diffusion of the stain. For permanent mounting a neutral, water-soluble mounting fluid called Abopon has been found satisfactory. This new method stains the hyphae pink, but leaves the cotton fiber practically unstained. It has been used successfully with a number of fungi present on cotton fibers and fabrics, and may be found valuable for other purposes.

Method of Calculating and Presenting the Data

The results of the physical and chemical tests are presented in the form of graphs which show the percentage of change for each observation from its control. Each graph represents the data for any one test with any one organism. The percentage of change for each sample from its control is plotted as a point on a graph and the mean of the replicates as a cross. Thus in each graph each period of incubation is represented by one cross and a series of points equal to the number of replicates. The value for each treated sample is compared with its specific control, since the fabric was sampled in such a way that each test piece was taken from the immediate vicinity of its control.

An equation was fitted to the data by the method of least squares. This equation is plotted on each graph as a solid line. The value for

the percentage change at zero day's incubation was calculated from the equation. The portion of the curve connecting this point with the first observed period of incubation is represented by a dotted line.

RESULTS

Appearance of Fabric

The appearance of the fabrics incubated with the micro-organisms can be illustrated best by a series of photographs. The growth of *Chaetomium globosum* and of *Spirochaeta cytophaga* on squares of fabric are shown in figure 2. The surface of the cloth treated with *Ch. globosum* (fig. 2. *B*) is covered with the perithecia of the fungus

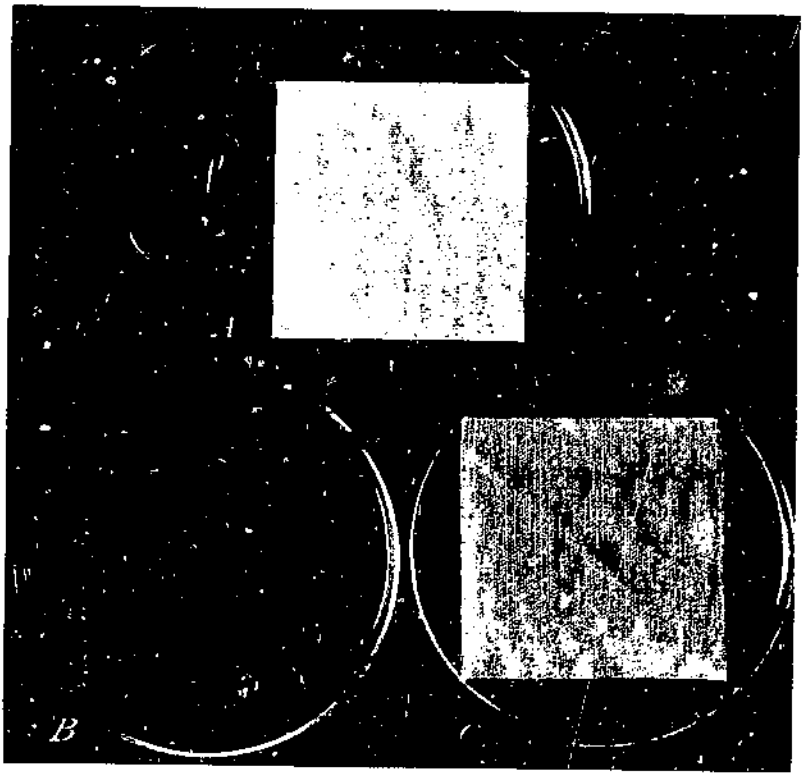


Figure 2.—Squares of duck incubated on agar medium in Petri dishes for 15 days: *A*, in a sterile condition; *B*, with *Chaetomium globosum*; *C*, with *Spirochaeta cytophaga*.

to such an extent that the fabric is no longer visible. The color of this growth is dark grayish green, and its rough surface is apparent from the photograph. The growth of *S. cytophaga* (fig. 2. *C*), on the other hand, does not obscure the weave of the duck, is yellow in color, and gelatinous in appearance.

The fabric itself changed in color from white to light tan as growth of the chaetomium progressed, and from white to a yellowish white

as the spirochaete developed. These color comparisons were made visually after the surface growth had been removed with a spatula and the test pieces rinsed in water and dried. Even after 15 days' exposure the deteriorated fabrics appeared to be in a good condition. In no case was the extent of damage indicated by the appearance of the fabric.

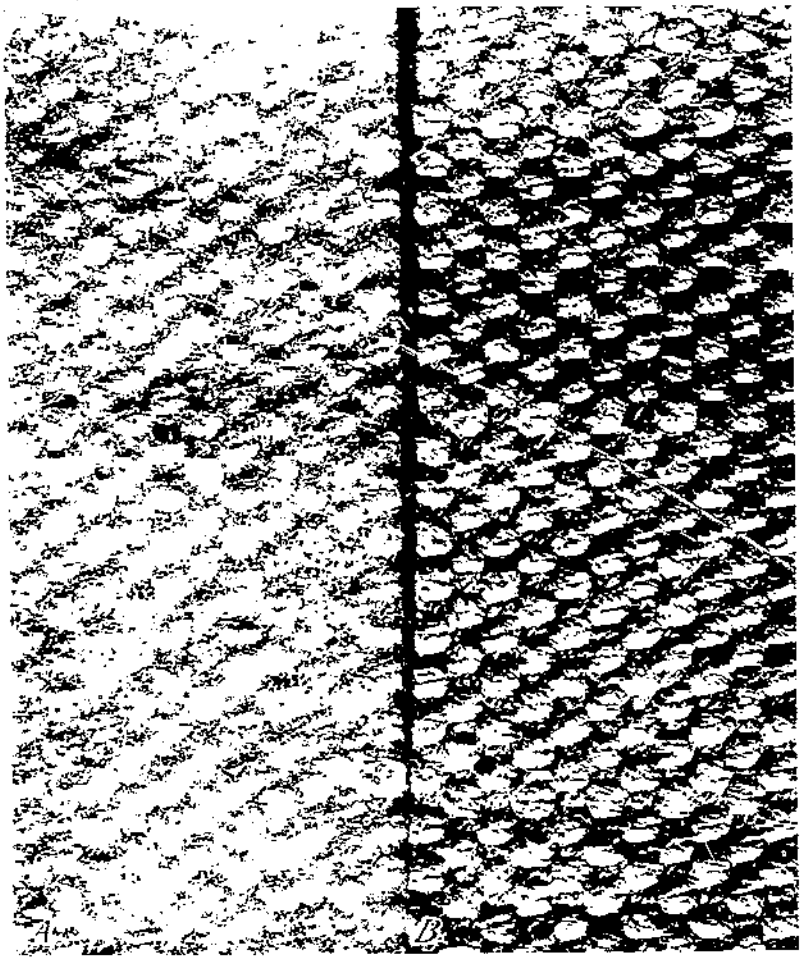


FIGURE 3.—Photomicrograph of duck; *A*, untreated fabric; *B*, fabric incubated for 15 days with *Chaetomium globosum*. ×10

Figure 3, *A*, is a photomicrograph of the untreated cotton duck, and 3, *B*, of the same material after 15 days' incubation with *Ch. globosum*. The weave of the cloth appears more distinct in the treated material than in the control since the protruding fibers in the former have been broken off as a result of the action of the microorganism. When fibers are removed from the fabric and examined under a higher magnification than was used for the photomicrograph,

the ends of the fibers from the incubated material are seen to be jagged, while those from the untreated fabric are unbroken. Apparently the fungus is more active on the side of the fabric exposed to the air than on the side placed next to the agar, since the latter is similar in appearance to the untreated duck shown in figure 3. When fabrics incubated with *S. cytophaga* were examined microscopically as described above, similar results were observed.

Analysis of Controls

The physical measurements give the following average values for the duck fabric used in this study: Warp breaking strength, 133.6 pounds; filling breaking strength, 141.7 pounds; weight, 13.6 ounces per square yard; and thickness, 0.031 inch. The upper quartile length of the fibers removed from the warp yarns of the duck for the staple length measurements is 0.937 inch, and of the fibers from the filling yarns, 0.906 inch.

Results of the chemical tests are: Fluidity, 13.00 reciprocal poises; copper number, 0.39; methylene blue absorption, 0.99; moisture, 6.35 percent; and ash, 0.04 percent.

The breaking-strength values are the average of 112 individual controls; weight, thickness, and ash, of 56 controls; fluidity, copper number, and methylene blue absorption, 42 controls; moisture, 28 controls; and staple length, 8 controls.

Breaking Strength

The breaking strength of the cotton duck was reduced by the action of both the fungus and the bacterium. As incubation continued, the strength decreased progressively.

Figure 4 shows the percentage decrease in warp and filling breaking strength, and illustrates the method adopted for presenting the data. The points plotted show the percentage change of each breaking-strength value from its control, and the crosses, the mean of these individual points for any given period. Each of these means is the average of 8 individual values.

When the percentage loss in breaking strength is plotted against time of incubation, the resulting curves (fig. 4) are found to be similar in form to the well-known growth curves. A typical growth or population curve was described by Pearl (*23*) for the rate of growth of a population of yeast cells and by Buchanan and Fulmer (*8, p. 36*) for the rates of growth of bacteria. In these curves, the population rate increases until it attains a maximum, after which it decreases until a practically uniform rate is reached, when the population becomes stationary.

These curves are the type known as logistic curves and are defined by the general equation $y=\frac{K}{1+e^{a+bt+ct^2}}$.

In the present study, y equals the percentage of loss of breaking strength; K equals 100, i. e., complete loss of breaking strength in percent; e equals the base of the natural system of logarithms, i. e., 2.7183; t equals time of incubation in days; and a, b, and c are con-

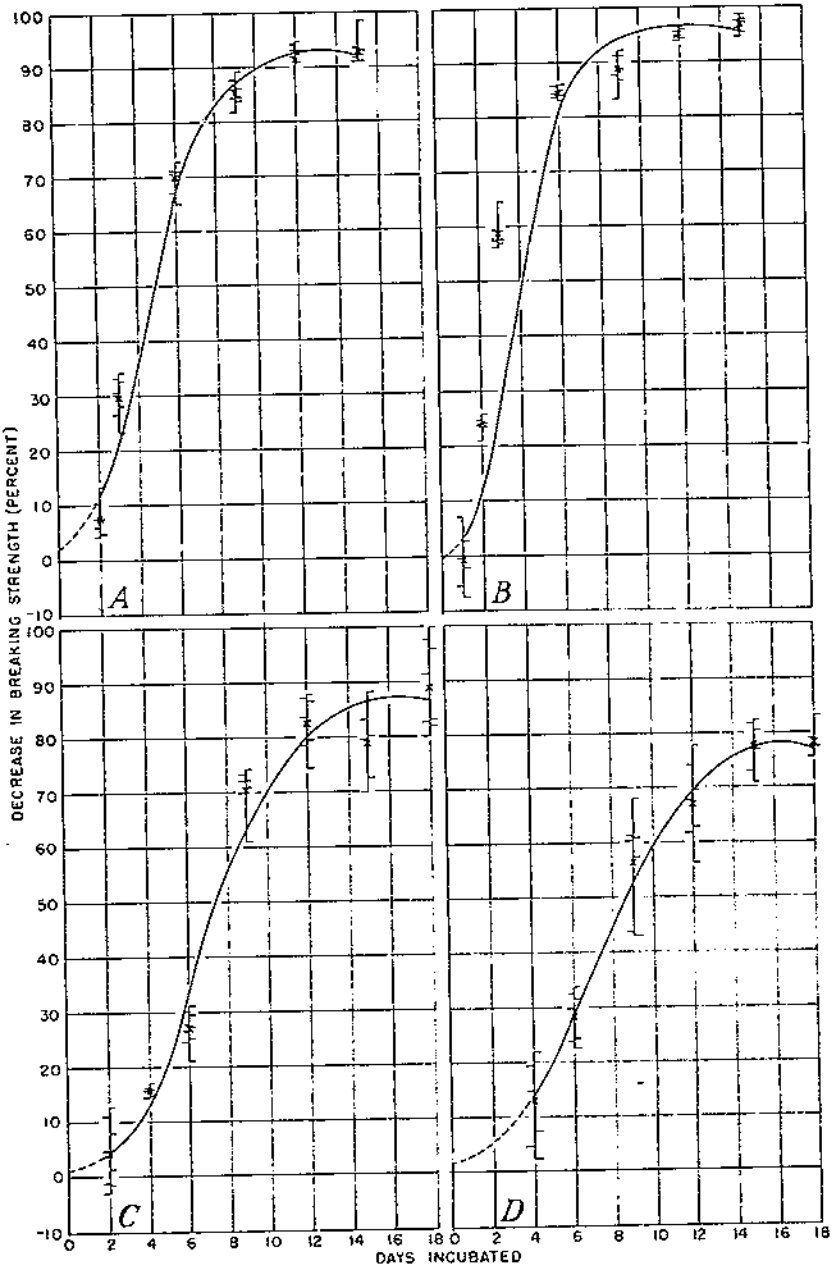


FIGURE 4.—Percentage decrease in breaking strength of inoculated cotton duck incubated for various lengths of time: *A*, warp direction, *Chaetomium globosum*; *B*, filling direction, *Ch. globosum*; *C*, warp direction, *Spirochaeta cytophaga*; *D*, filling direction, *S. cytophaga*.

stants the values for which are calculated for each set of data. The equation found for the warp breaking-strength data for the material incubated with *Ch. globosum* is $y=\frac{100}{1+e^{3.9002-0.9849t+0.0373t^2}}$ and for the filling data $y=\frac{100}{1+e^{4.0776-1.2134t+0.0497t^2}}$

The warp and filling breaking strength equations for *S. cytophaga* are

$$y=\frac{100}{1+e^{4.5512-0.7780t+0.0234t^2}}$$

and

$$y=\frac{100}{1+e^{4.0767-0.6930t+0.0186t^2}}$$

respectively. For each equation, the constants a and c have positive values and b, negative. The respective values for a, b, and c do not differ appreciably. Therefore the curves (fig. 4) representing these equations also are similar.

Figure 4 shows that in general the rate of loss of strength is greatest between the second and ninth days of incubation. For example, the daily loss in strength warpwise for the duck treated with the spirochaete is approximately 2.0 percent for the first 2 days, 9.5 percent for the next 7 days, and 2.1 percent for the last 9 days.

The rate of loss of breaking strength is more rapid for the chaetomium than for the spirochaete. This is indicated by the slopes of both the lag phase and the logarithmic phase of the curves. After incubating the fabric for 6 days with the fungus, the loss in filling strength is 84.5 percent, and with the bacterium, 28.4. This disparity may be due to the difference in the rate of growth during the lag phase. The rapidity with which *Ch. globosum* attacked cellulose under the conditions of this study is illustrated by the fact that the fabric lost 58.7 percent fillingwise after only 3 days' incubation with this fungus.

After 15 days' exposure the duck treated with the chaetomium lost 93.1 percent in strength warpwise and 96.9 percent fillingwise. This conforms with the finding of Thom, Humfeld, and Holman (*35*), who reported a 94.5 percent loss in warp strength for a duck fabric incubated 14 days with *Ch. globosum*. The warpwise and fillingwise losses for the spirochaete are 79.1 and 77.6 percent, respectively.

Searle (*28*) found a loss in filling breaking strength of approximately 70 percent when he inoculated a duck fabric of American cotton with a soil suspension and incubated the fabric for a period of 6 weeks. Heyes and Holden (*18*) reported a loss in breaking strength of from 13 to 86 percent when yarns of American cotton were incubated for 3 months with several species of *Penicillium* contaminated with bacteria. In these last two investigations, the rate of break-down was much slower than in the present study. The rapid rate reported here may be due to the fact that the experimental conditions more nearly approached the optimum for the growth of the organisms, or to the more destructive action of *Ch. globosum* or *S. cytophaga* as compared with the mixed cultures of soil organisms used by Searle or the *Penicillium* species investigated by Heyes and Holden.

Figure 4 shows that the chaetomium had a greater effect on the filling strength of the fabric than on the warp strength. On the other hand, the spirochaete which forms no hyphae, reduced the warp strength more than the filling.

WEIGHT

Incubation of the duck with *Ch. globosum* and with *S. cytophaga* produced a decrease in weight. In general, the fabric lost weight progressively as the length of treatment with each organism continued.

In figure 5 the points and crosses have the same significance as in figure 4. The curve plotted in figure 5, *A*, for the fungus was calculated by means of the second-degree polynomial equation $y=a+bt+ct^2$ and in 5, *B*, for the bacterium by the third-degree polynomial, $y=a+bt+ct^2+dt^3$. By adding the additional term to the second equation, the calculated curve was found to fit the observed data more closely. These polynomial equations can be used to fit parts of growth curves. For expressing the relationship between weight and age of chick embryos Snedecor (*33*) used a fourth-degree polynomial equation. Since no doubt the rate of loss of weight of the fabric is related to the rate of growth of the microorganisms it seems reasonable to use polynomial equations for expressing the relation between weight and length of incubation.

In these equations, y equals the percentage loss of weight; t, the incubation time in days; and a, b, c, and d are constants, the values of which were calculated from the data. For the fabric incubated with the chaetomium the equation was found to be $y=-5.5446+3.1844\ t-0.1014\ t^2$ and with the spirochaete $y=-2.7430+2.1203\ t-0.1889\ t^2+0.0074\ t^3$. In both equations the values for a and c are negative and for b, positive. The respective values for a, b, and c do not differ greatly from each other. Since these similar equations fit the data, it appears that the rate of change of weight follows some definite law and that the weight data for the two organisms are related.

The loss in weight is greater for the material when treated with the chaetomium than when incubated with the spirochaete. For example, after 9 days the samples exposed to the fungus lost 15.4 percent in weight and to the bacterium, 6.8 percent. The greater effect of *Ch. globosum* on the deterioration of the duck also is shown by the strength data.

The presence of hyphae in the fabric treated with the chaetomium may have influenced somewhat the decrease in rate of loss of weight toward the end of the period of incubation with the fungus. The hyphae have been observed to grow not only on the surface of the fibers but to penetrate the walls and may be found even in the lumen. The curve for the spirochaete shows that even after 18 days' incubation there is no decrease in rate of loss of weight.

When fibers taken from the fabric incubated with *Ch. globosum* were stained with basic fuchsin, the hyphae of the fungus took the pink stain readily, so that a clear differentiation was obtained against the unstained fiber background. Most of this contrast between fiber and hyphae is lost in a black and white photomicrograph, so that plate 1 does not give the clear picture that is obtained when the mate-

Fibers stained with basic fuchsin: *A*, Untreated fibers; *B*, fibers removed from the fabric incubated for 15 days with *Chaetomium globosum*. × 250.

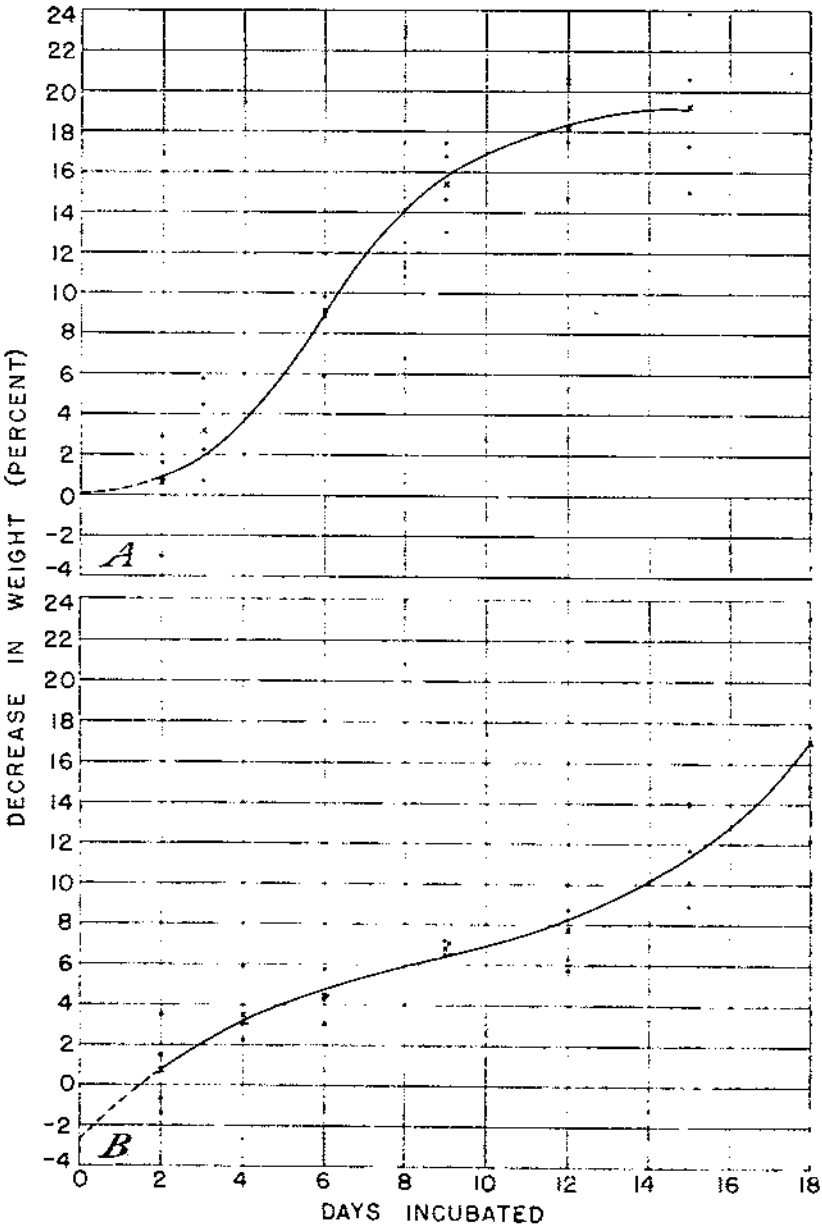


FIGURE 5.—Percentage loss in weight of inoculated and incubated duck: *A*, *Chaetomium globosum*; *B*, *Spirochaeta cytophaga*.

rial is viewed through the microscope. The hyphae are seen to be present on and in the individual fibers. They are very slender, measuring only about one-twentieth to one-tenth of the diameter of the cotton fiber, and form in places more or less of a network. The fibers removed from the fabric incubated with *S. cytophaga* are stained pink by the basic fuchsin in certain areas. When these areas are examined at a magnification of 900, the outlines can be seen of what may be the cells of the spirochaete, or a roughness of the surface of the fiber produced by the action of the organism. The untreated fibers do not show this phenomenon, nor do the fibers deteriorated by the chaetomium.

Thickness

Both organisms reduced the thickness of the fabric, as well as its strength and weight. The equation $y = -2.3177 + 2.1460\ t - 0.0943\ t^2$ was found to represent the percentage decrease in thickness for *Ch. globosum*, and $y = -3.6389 + 2.4435\ t - 0.2382\ t^2 + 0.0082\ t^3$ for *S. cytophaga*. As in the case of the weight data, a second-degree polynomial equation was found for the fungus and a third-degree for the bacterium. The corresponding constants in each equation have the same sign and are of approximately the same magnitude.

As shown in figure 6, loss in thickness is on the whole greater for the cloth treated with *Ch. globosum* than for that attacked by *S.*

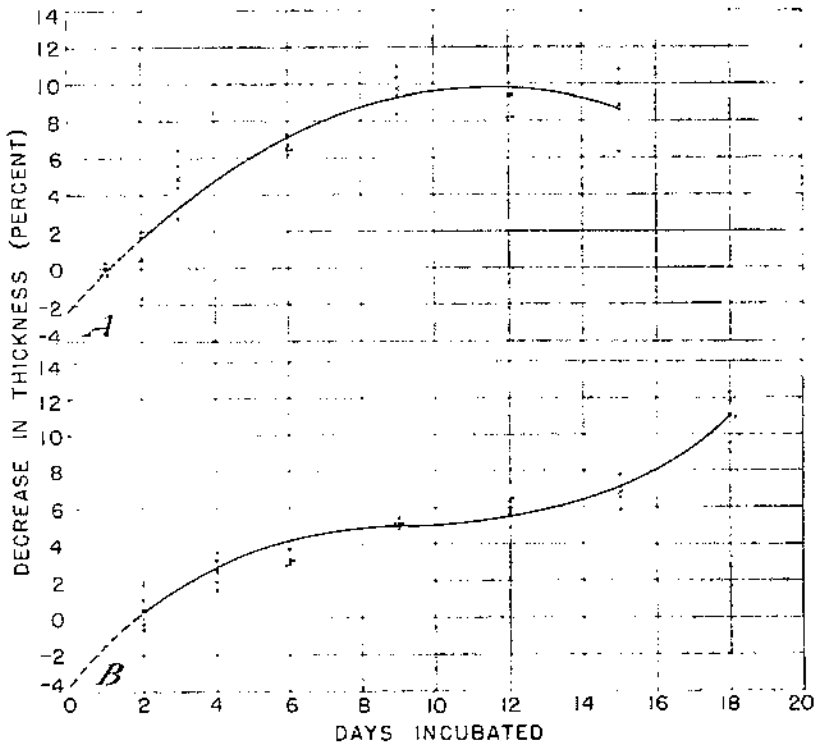


Figure 6.—Percentage decrease in thickness of duck produced by the action of the micro-organisms: *A*, *Chaetomium globosum*; *B*, *Spirochaeta cytophaga*.

cytophaga. In general, the thickness of the material treated with the bacterium that forms no hyphae decreases progressively as incubation continued, while the thickness of the samples treated with the hyphae-forming fungus remains approximately constant after 9 days' exposure. Possibly the accumulation of hyphae on and in the fibers of the cotton treated with *Ch. globosum* compensated, toward the end of the incubation period, for the loss in thickness of the fabric.

The curve in figure 6, *B*, for *S. cytophaga* is very similar to the curve for this organism representing changes in weight. This seems to indicate a close relationship between loss of weight and thickness for the fabric when deteriorated by bacterial action.

Staple Length of Fiber

Staple-length measurements were determined on fibers removed from the untreated fabric and from the fabric after 3, 6, and 9 days' incubation with *Ch. globosum* and after 6, 9, 15, and 18 days' incubation with *S. cytophaga*. Figure 7 shows that the length of the fibers decreases as a result of deterioration caused by both the fungus and the bacterium.

The effect of the chaetomium on the staple length of the fiber was very pronounced. The sample incubated for only 3 days showed on analysis a considerably greater proportion of short fibers than the control. After 6 and 9 days' incubation a large percentage (fig. 7, *A*, *B*) of the combed fibers are only three-sixteenths of an inch long; and after 12 days it was impossible to make an accurate measurement of staple length, since the fibers were too short. This decrease in staple length is no doubt closely related to the practically complete loss of breaking strength.

The distribution of the fiber lengths in the warp and filling yarns of the unincubated duck is very similar (fig. 7). However, after 3 days' incubation with *Ch. globosum*, approximately 19 percent of the fibers from the filling yarns are one-sixteenth of an inch long, whereas only 4 percent of the fibers from the warp yarns are of this length; after 6 days these values are 24 and 9 percent, respectively; and after 9 days, 42 and 19 percent. The loss in breaking strength resulting from the growth of the chaetomium also is greater fillingwise than warpwise.

Deterioration caused by the bacterium had less effect on the staple length of combed fibers than damage produced by the fungus. The decrease in length after 6 days' treatment with the spirochaete was so slight that no measurements were made on the material incubated for 2 or 4 days. The fabric when incubated for 18 days with *S. cytophaga* was less deteriorated as measured by staple length than when treated for 9 days with the fungus. The bacterium also had less effect on the breaking strength of the fabric than did the fungus. As with *Ch. globosum*, the staple length of the fibers from the filling yarns are affected more than the fibers from the warp yarns.

The upper quartile length of the fibers removed from the warp yarns of the untreated duck is 0.937 inch, and from the filling yarns, 0.906 inch. This measurement for the fibers removed from the warp yarns of the duck incubated with the chaetomium for 3, 6, and 9 days is 0.596, 0.372, and 0.282 inch, respectively. The corresponding values

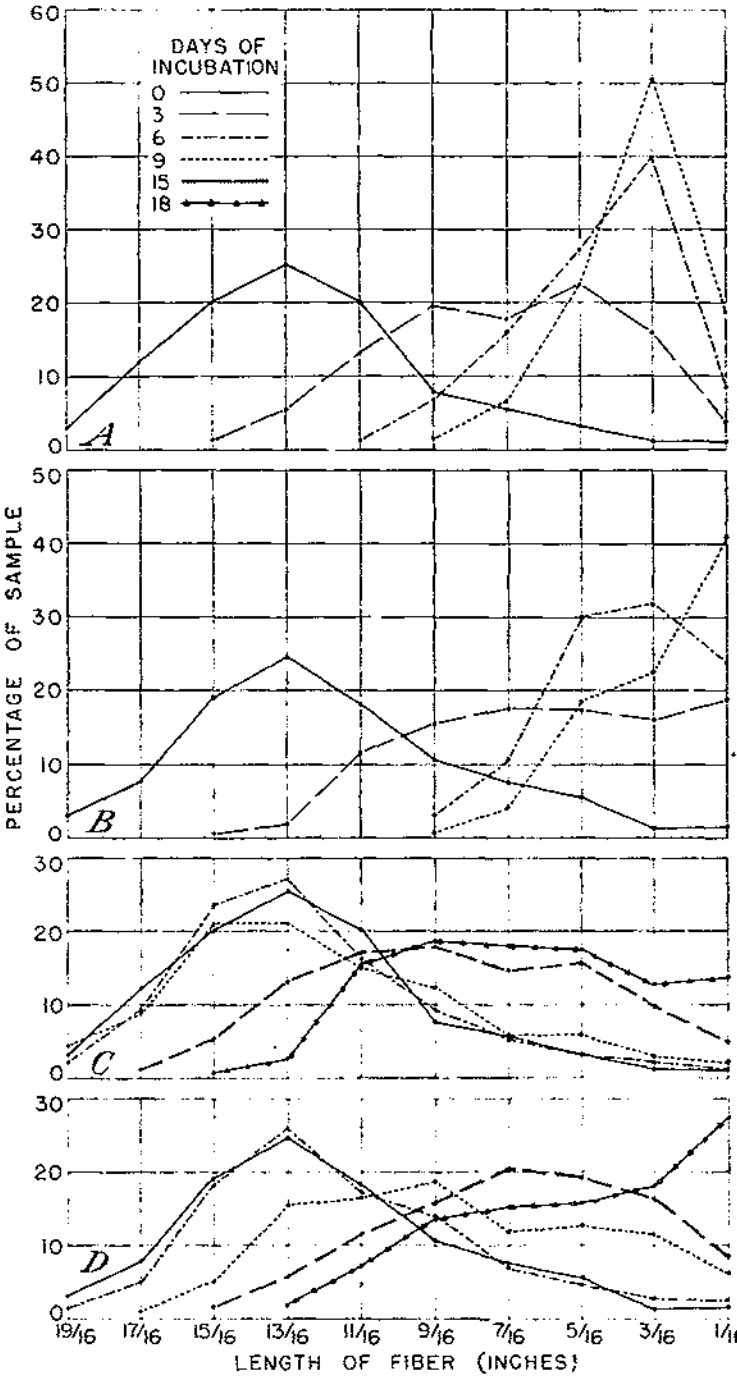
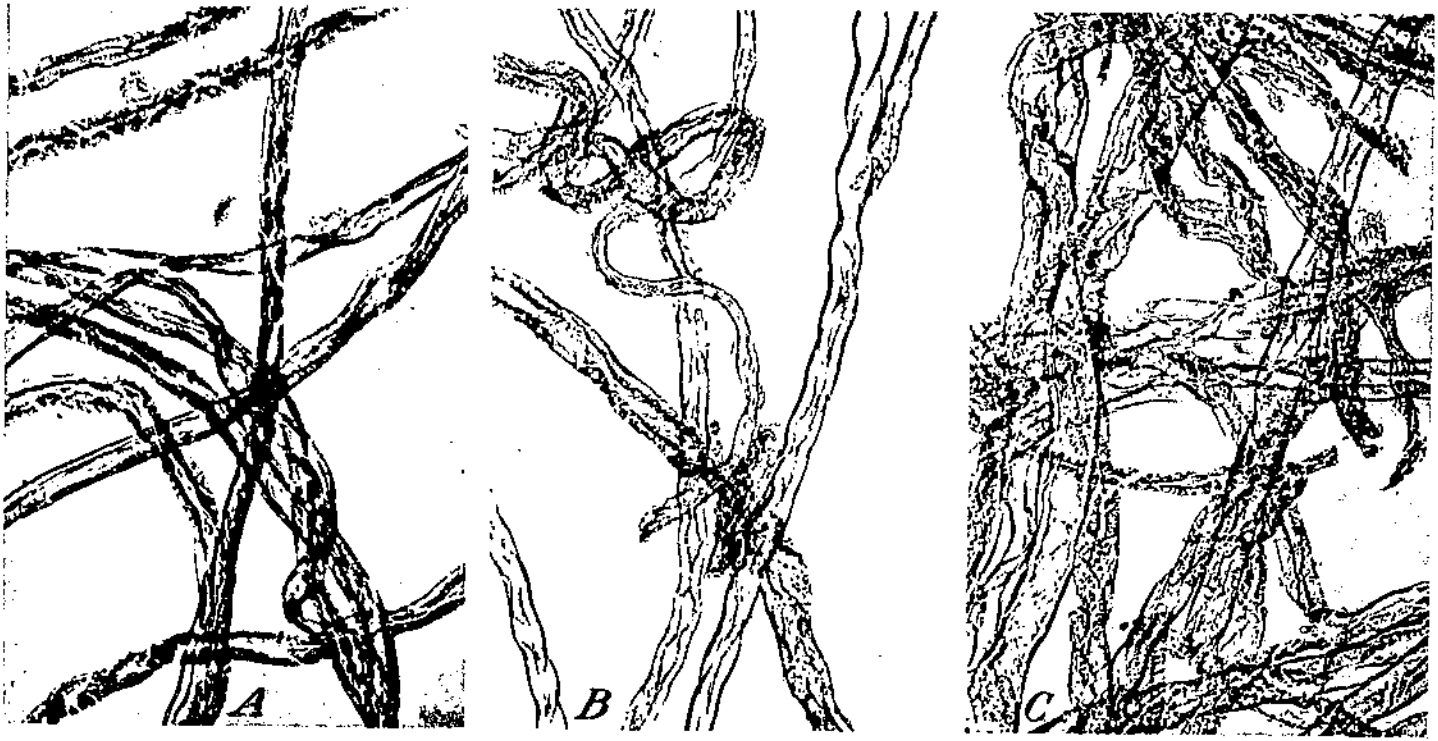


FIGURE 7.—Staple length of fibers in the duck after inoculation and incubation for various periods of time: *A*, Warp direction, *Chaetomium globosum*; *B*, filling direction, *Ch. globosum*; *C*, warp direction, *Spirochaeta cytophaga*; *D*, filling direction, *S. cytophaga*.



Photomicrographs of fibers treated with Fleming and Thaysen solution: *A*, Untreated fibers; *B*, fibers removed from fabric which was incubated for 3 days with *Chaetomium globosum*; *C*, fibers from fabric incubated for 15 days with *Ch. globosum* × 175.

for the filling yarns are 0.540, 0.328, and 0.252 inch. The fiber lengths for the spirochaete treated warp yarns after 6, 9, 15, and 18 days' incubation are 0.928, 0.930, 0.712, and 0.586 inch, and for the filling yarns, 0.878, 0.729, 0.585, and 0.486 inch.

The fibers deteriorated by the action of *Ch. globosum* for 15 days are too short to be sorted. Also there is an almost complete loss in breaking strength, although the changes in appearance, weight, and thickness are not large. For *S. cytophaga*, also, the changes in staple length and breaking strength are greater than those in appearance, weight, and thickness.

When the cotton fibers that had been removed from the fabric incubated with the chaetomium were examined under the microscope, it was noted that the hyphae of the fungus frequently had penetrated the walls and apparently were breaking down the cellulosic constituents of the fibers. In some instances, the hyphae were growing in the lumen. This probably weakened the individual fibers to such an extent that they broke when the fabric was raveled and the yarns untwisted to make the staple-length determinations, even though the fabric samples and yarns were handled as carefully as possible.

The fibers of the fabric incubated with the spirochaete, on the other hand, did not show any such penetration. Instead, the organisms seemed to surround the fibers and as growth progressed, gradually to use up the outside cellulose wall. This would weaken the fiber along its whole length rather than at any one place. There was, therefore, much less tendency for the fibers to break and the percentage of short fibers did not increase to the same extent as for the material attacked by the chaetomium.

Some of these fibers were treated with Fleming and Thaysen solution (*12*), a mixture of carbon disulfide and sodium hydroxide, and then examined under a magnification of 175. Plate 2, *A*, is a photomicrograph of the fibers before incubation; *B*, after 3 days' incubation with *Ch. globosum*, and *C*, after 15 days. The change from the undamaged to the deteriorated condition is visible after only 3 days. In the fibers incubated for 15 days, the total breakdown of the cell walls is striking. The destructive action of *Ch. globosum* was more rapid than that of *S. cytophaga*. For example, when the fabric incubated with the spirochaete was treated in a similar manner with the Fleming and Thaysen solution, the fibers after 15 days' incubation with the spirochaete seemed to show about the same degree of deterioration as those with the chaetomium after only 3 days.

Fluidity

Figure 8 shows that in general the values for fluidity, which are a measure of chemical deterioration, increase as incubation with *Ch. globosum* and with *S. cytophaga* continued. The percentage increases in fluidity are all small and much less than those observed when the breaking strength of the fabric is reduced to the same extent by acids or chemical oxidizing agents.

The equations found for the fluidity data are

$y=-0.2583+0.7658t-0.0191t^2$ for the chaetomium and
$y=-1.5271+0.9049t-0.0298t^2$ for the spirochaete.

These results do not agree with the findings of Searle (*28*) who obtained no increase in fluidity when he incubated duck made from American cotton with a mixture of soil organisms. He states that "the weakening effect of micro-organisms on fibers is not such as brings about a degradation of the cellulose in the chemical sense." More recently, however, Heyes and Holden (*18*) obtained an increase in fluidity when a yarn of American cotton was inoculated and incubated with a culture of a species of *Penicillium*.

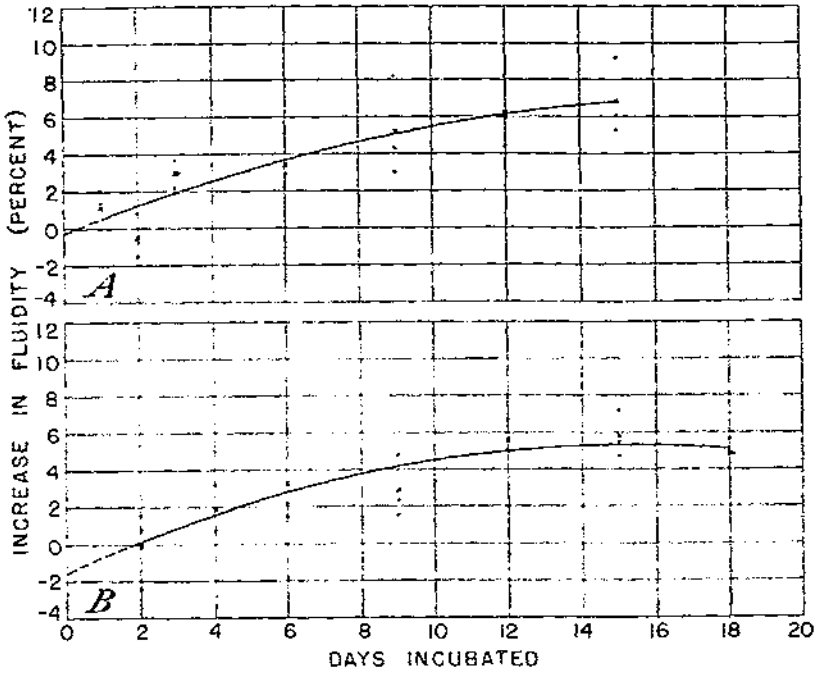


FIGURE 8.—Percentage increase in the fluidity values of cuprammonium solutions of the fabric tested after various periods of incubation with the organisms: *A*, *Chaetomium globosum*; *B*, *Spirochaeta cytophaga*.

COPPER NUMBER

The copper-number value of a cotton cellulose varies directly with the amount of degraded cellulose of an aldehydic or ketonic structure present. It, therefore, is one measure of the chemical deterioration of cellulose.

Figure 9, *A*, shows that the percentage of change in the copper-number values of the material incubated with *Ch. globosum* becomes larger as the treatment progressed. This rise in copper numbers is smaller than is usually found when breaking strength is reduced almost completely.

The curve showing the percentage of increase in the copper numbers indicates that this change is related to the rate of loss of breaking strength. It is a logistic curve of the form $y=\frac{K}{1+e^{a+bt+ct^2}}$ as found for breaking strength. For the copper-number data, $K=77.2$, which

value can be compared with a total loss of breaking strength of the fabric. The equation which fits the data is

$$y=\frac{77.2}{1+e^{5.5084-1.3710t+0.0384t^2}}.$$

The rise in copper number found for the material incubated with the chaetomium agrees with the results of Heyes and Holden (*18*) who reported increased copper numbers for a cotton yarn attacked by various species of *Penicillium*.

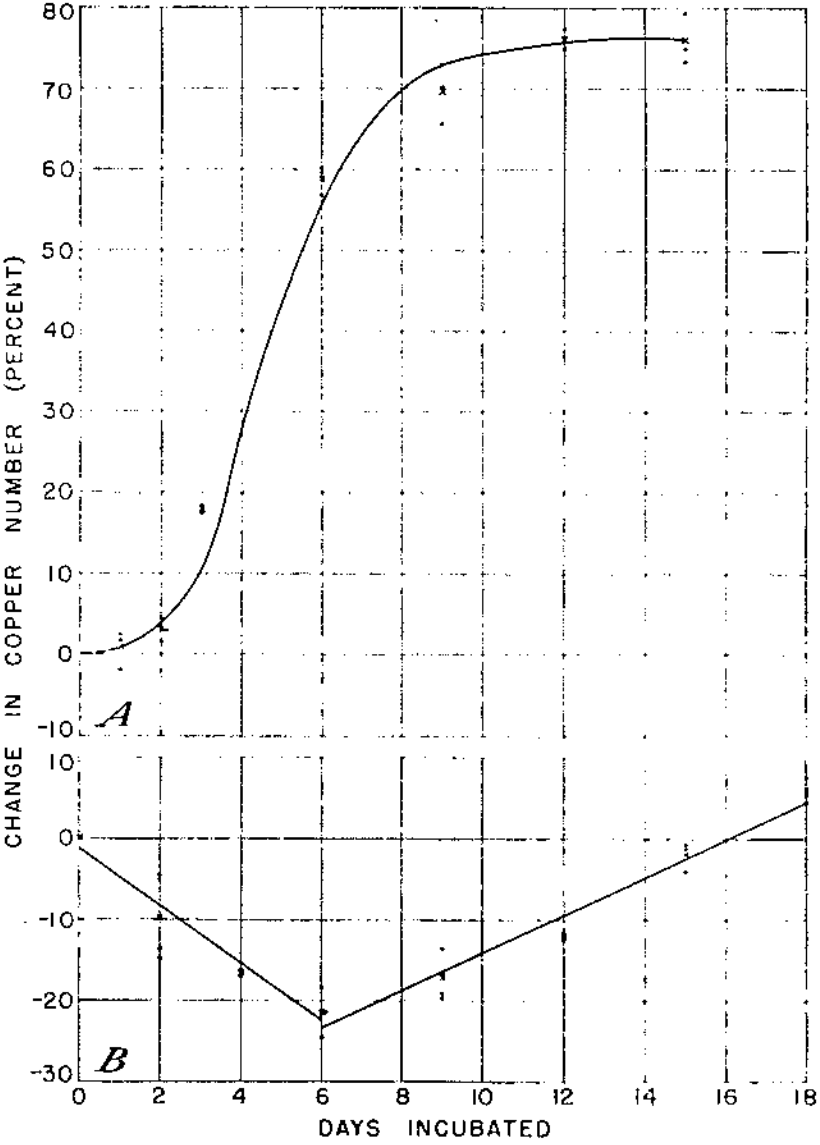


FIGURE 9.—Percentage change in the copper-number values of duck attacked by the micro-organisms: *A*, *Chaetomium globosum*; *B*, *Spirochaeta cytophaga*.

The growth of *S. cytophaga* on the fabric appears to have an entirely different effect on the copper number (fig. 9, *B*). In this case the copper number decreases at a uniform rate until the sixth day, after which it increases at an approximately similar rate until at 18 days it is about 6 percent greater than at the beginning of the incubation period. Since the copper numbers at first are reduced, it is possible that the bacterium attacked degraded cellulose of an aldehydic or ketonic structure before it used undamaged cellulose. After the sixth day, the rate of growth may have been so great that the undamaged cellulose was broken down at a faster rate than the compounds characterized by high copper number were utilized. Searle (*28*) noted a decrease in the fluidity values of cotton fabrics incubated with mixed cultures. Apparently in his experiments the organisms also attacked the degraded cellulose first. The equation for the line representing the change in copper number for the first 6 days is $y=1.1903-3.5449t$ and for the last 12 days is $y=-37.1600+2.3200t$.

Methylene Blue Absorption

The methylene blue absorption test measures the amount of oxidized cellulose formed characterized by carboxylic groupings. Figure 10 shows that the rate of formation of this type of oxidized cellulose is greatest during the early stages of incubation. It gradually decreases for both species of organisms and becomes practically constant after 12 days. After 15 days the percentage increase in absorption for the fabric incubated with *S. cytophaga* is approximately 40 percent and with *Ch. globosum*, approximately 60 percent. Since the absorption value for the untreated fabric is small, the actual amount of methylene blue absorbed is not appreciable in either case.

The relationship between methylene blue absorption and time of incubation is expressed by a second-degree polynomial. The equation $y=-5.4917+8.1701t-0.2574t^2$ was found for the fabric treated with the chaetomium and $y=-10.9446+7.1006t-0.2363t^2$ with the spirochaete. The similarity between the equations is shown graphically in figure 10. The changes found for fluidity, copper number, and methylene blue absorption show that there is chemical deterioration and refute Searle's statement (*28*) that cellulose is not deteriorated in a chemical sense by the action of micro-organisms.

Moisture

Figure 11 shows that toward the end of the period of incubation with *Ch. globosum*, the moisture content increased about 10 percent. The change in the percentage of moisture of the fabric when incubated with *S. cytophaga* proceeds more gradually and at a more uniform rate than that with the chaetomium. After 18 days the material treated with the fungus has a moisture content of approximately 9 percent.

The equation $y=1.9172+1.2927t-0.0511t^2$ was found for the chaetomium data and $y=0.2836+0.5452t-0.0004t^2$ for the spirochaete.

Burgess (*9*) found that the moisture content of wool increased when he incubated strips of inoculated wool. He ascribed this rise

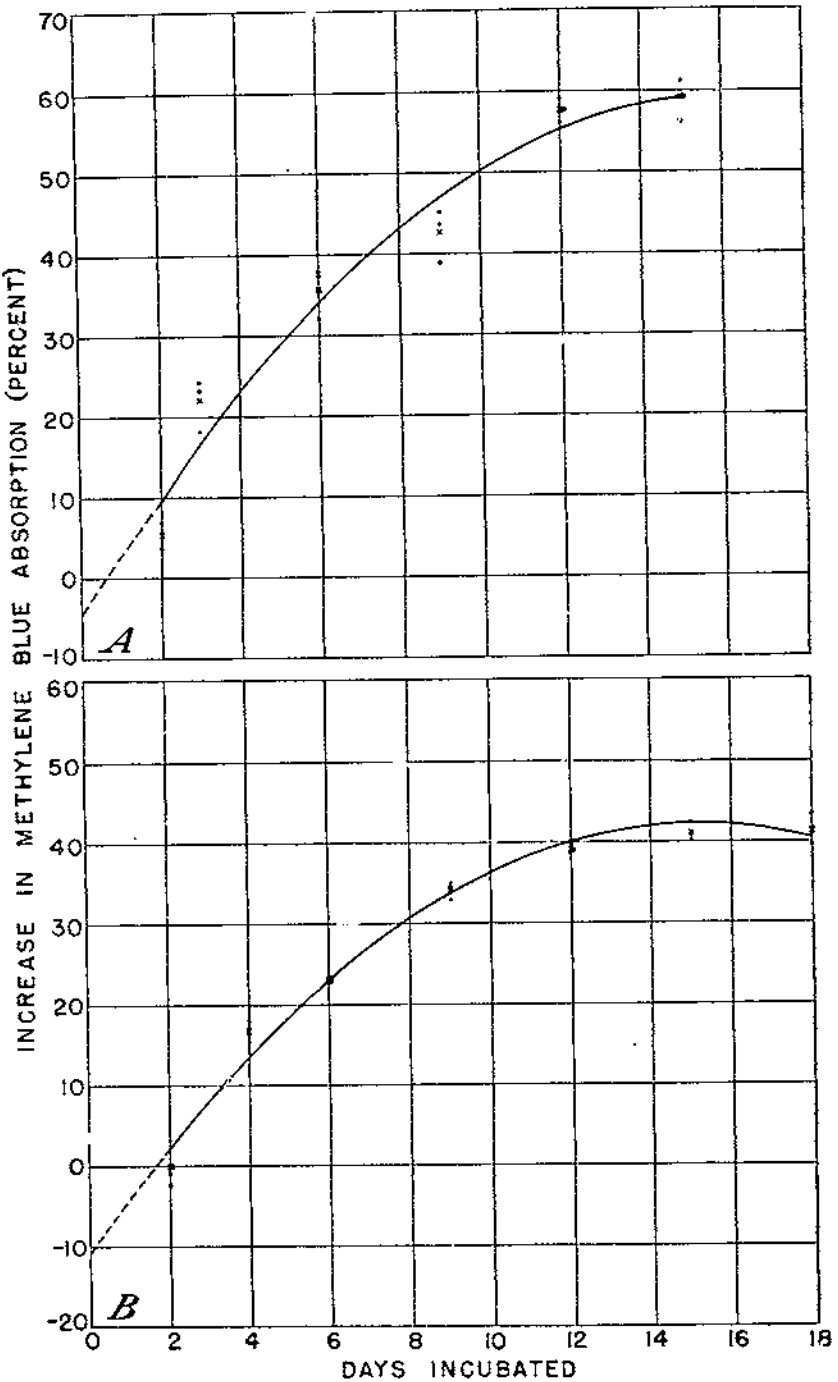


FIGURE 10.—Percentage increases in the methylene blue absorption of duck tested after various periods of incubation with the organisms: *A*, *Chaetomium globosum*; *B*, *Spirochaeta cytophaga*.

in moisture to condensation caused by small changes in temperature during incubation. It seems entirely possible, however, that the moisture increase was a result of microbiological attack.

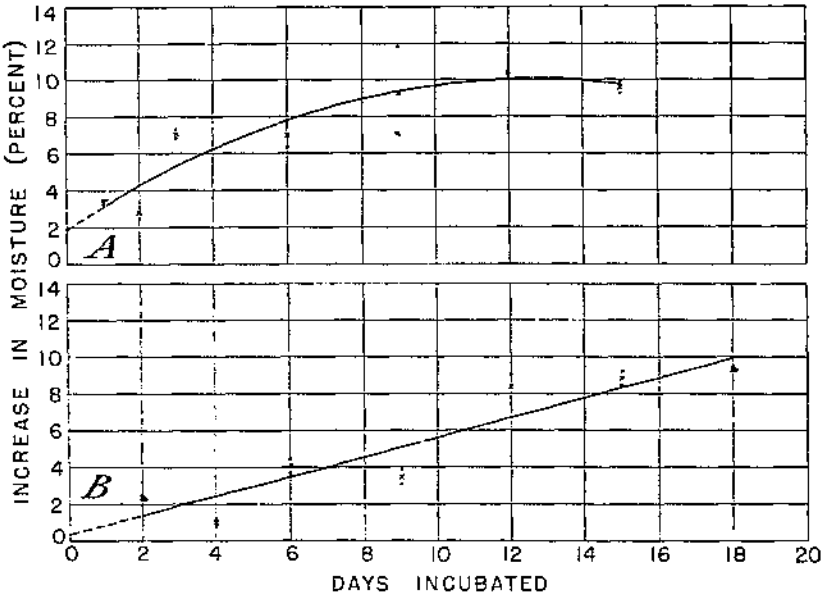


FIGURE 11.—Percentage gain in moisture of inoculated and incubated duck: *A*, *Chaetomium globosum*; *B*, *Spirochaeta cytophaga*.

ASH

The ash content of the fabric increased during incubation with both organisms (fig. 12). The rise in this value is greater for *Ch. globosum* than for *S. cytophaga*. For example, after 15 days' incubation the increase in the ash content of the duck deteriorated by the fungus is 116 percent and by the bacterium, 77 percent. Although the percentage of increase in ash is large, the actual increase is very small, since the ash content of the untreated material is only 0.04 percent. The individual values for ash varied considerably at each test period (fig. 12), but this is not surprising when the small original ash content of the fabric is considered.

The progressive increase in the ash content of the fabric as exposure to the two cultures of organisms continued may be explained in part by assuming that the micro-organisms utilized only the organic portion of the cotton fiber but left the mineral content essentially unchanged. The percentage of ash in the residual fabric would then become larger as observed. This will not account for the total gain, however, since the percentage of increase in ash is approximately five times as great as the percentage of loss in weight.

Some of the increase may be due to the absorption of salts from the media. The ash content of the material incubated with *Ch.*

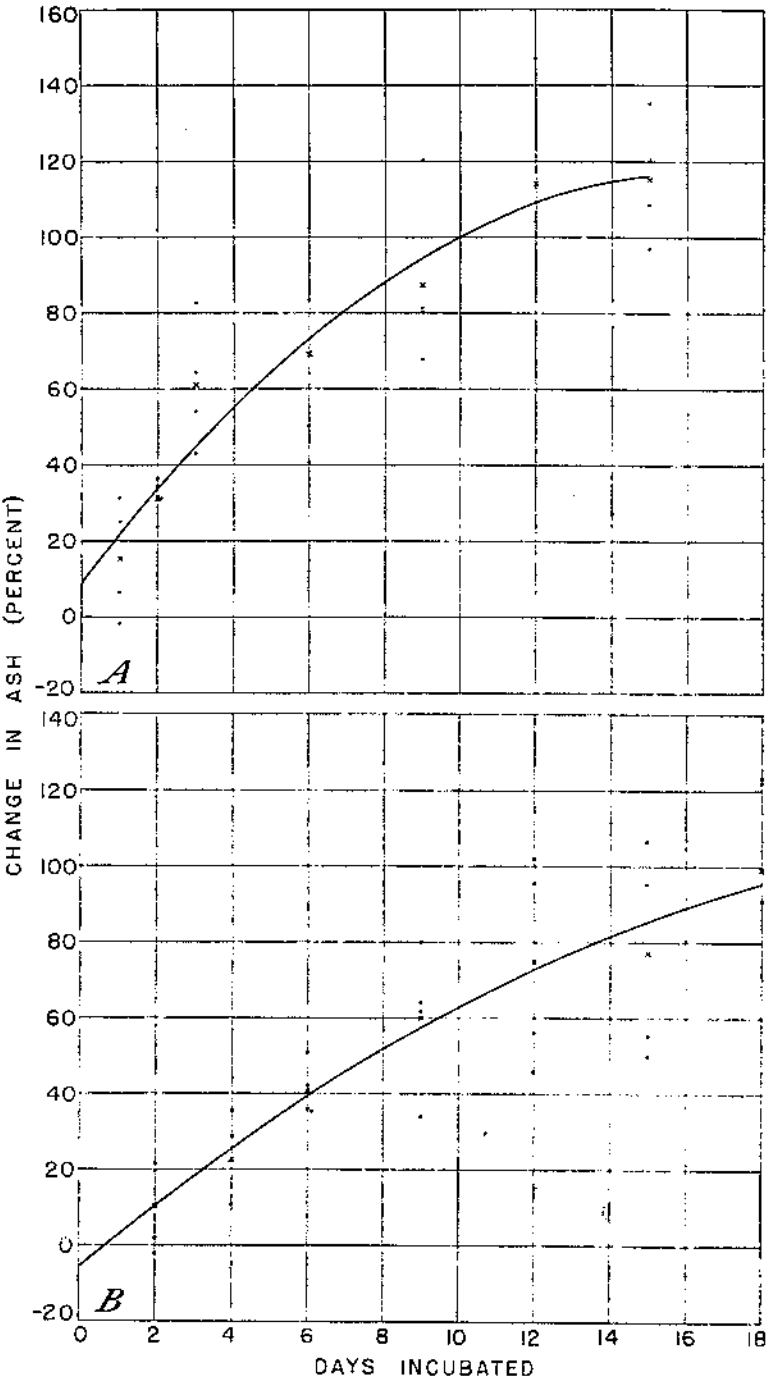


FIGURE 12.—Percentage increase in the ash content of duck incubated for various lengths of time with the micro-organisms: *A*, *Chaetomium globosum*; *B*, *Spirochaeta cytophaga*.

globosum increased more than that with *S. cytophaga*. The former material also was deteriorated to a greater extent than the latter as shown by all the physical and chemical tests. It seems possible, therefore, that the increased action of the fungus was responsible for the increased absorption of salts by the material treated with the fungus, and it is suggested that either the degraded material, the microbiological matter formed during incubation, or both, had a greater absorption capacity for salts than the original fabric. It is well known that such is the case with organic matter in soil. If the increased absorption of salts with incubation was due merely to contact of the undamaged fabric with the agar, the absorption in both cases would have been approximately the same. It is also possible, of course, that the decomposition products formed by the chaetomium had a greater absorptive power than those produced by the spirochaete. The absorbed salts apparently were fixed in the material in such a way as to be no longer water-soluble. At least they were not removed by the washing that all the fabrics received before the chemical analyses.

The equations that fit the data presented in figure 12 are

$y = 9.4453 + 13.0539t - 0.3952\ t^2$ for *Ch. globosum* and
$y = -5.8026 + 8.4496t - 0.1579\ t^2$ for *S. cytophaga*.

Carbon Dioxide Evolution

The rate of change in the various physical and chemical properties of the duck during incubation appears to be related to the rate of growth of the micro-organisms. Usually the rate of growth of micro-organisms is measured by determining the increase in their weight, the increase in their numbers, or the rate of evolution of carbon dioxide during their growing period. Aerobic organisms consume oxygen and respire carbon dioxide as they grow.

In this study an estimation of the increase in weight was not feasible, since it would be impossible to separate quantitatively the organisms from the fabrics. A fungus colony develops from a germinated spore into a mass of hyphae. When an attempt is made to determine numbers of organisms by plating out a suspension of the fungus, the hyphae are broken up into more or less irregular sections, each of which is capable of producing a fungus colony on the agar plate. Thus the resulting count is not a true estimate of the extent of growth. For the same reason a microscopic examination for determining numbers of these organisms is not trustworthy. Also, at present no method has been developed for counting numbers of cells of *S. cytophaga*, as this organism does not produce colonies on plating out on agar. A determination of the rate of carbon dioxide evolution was chosen, therefore, as the best measure available for the study of the rate of growth of the chaetomium and the spirochaete.

The rates of evolution of carbon dioxide during incubation of the duck with the organisms are shown in figure 13. The crosses in *A* and *B* represent the mean of four replications and in *C* and *D*, the mean of two. The other symbols in *B* and *D* indicate individual determinations. The amounts of carbon dioxide evolved on different days from the same strip of fabric are represented by the same symbol.

The curve in figure 13, *A*, which gives the milliequivalents of carbon dioxide given off each day per gram of fabric incubated with the fungus, does not follow the usual path of a growth curve. There is decrease in carbon dioxide evolution from the third to the seventh day. After the seventh day, the rate that had prevailed up to the third day is resumed, and a maximum rate is attained at about the tenth day. After this the carbon dioxide evolution takes place at a fairly uniform rate to the end of the experiment. The period of decreased carbon dioxide evolution coincides with the observed period

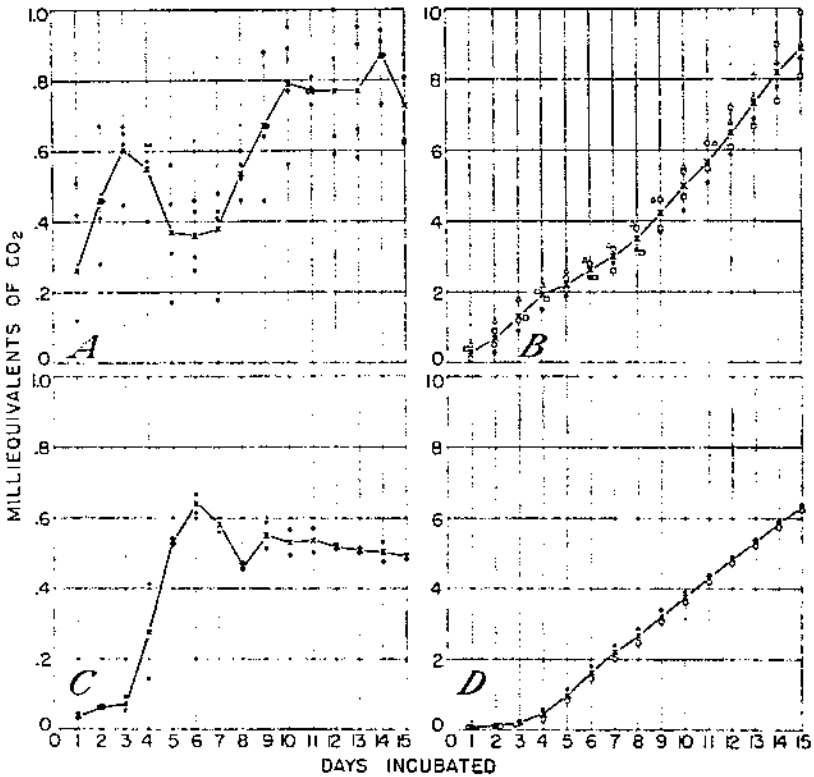


FIGURE 13.—The milliequivalents of CO_2 evolved per gram of duck during incubation with the organism: *A*, *Chaetomium globosum*, daily rate of evolution of CO_2; *B*, *Ch. globosum*, accumulated CO_2; *C*, *Spirochaeta cytophaga*, daily rate of evolution of CO_2; *D*, *S. cytophaga*, accumulated CO_2.

of perithecium and spore formation. Apparently during this period either the carbon dioxide or a portion of the intermediary products of cellulose degradation were utilized for this process. As soon as the perithecium and spore formation was completed, the carbon dioxide evolution was resumed at the rate observed previously. This decrease in rate occurred with all of the four sample strips, the first two of which were run concurrently, and then followed by two more in order to confirm this rather unexpected observation. It is believed that this interesting phenomenon has not been recorded previously.

Figure 13, *B*, shows the accumulated amount of carbon dioxide given off during the period of incubation with the fungus. The amount of carbon dioxide given off each day was added to the amount evolved on previous days. By plotting these accumulated values, a rather smooth curve is obtained, except for the period during the perithecium and spore formation.

The results for *Spirochaeta cytophaga* follow a fairly regular growth curve when the daily amounts of carbon dioxide evolved per gram of duck are plotted as shown in figure 13, *C*. The values for the first 3 days correspond to a definite lag phase. From the third to the sixth day there is a rapid rate of increase corresponding to a logarithmic growth phase and after the sixth day the rate, in general, becomes less. The values from the ninth to the fifteenth day correspond to the stationary phase of growth.

The sharp decrease after the sixth day indicates that growth was checked suddenly. Probably growth was inhibited when the pH value of the agar medium reached a certain value. The pH value rises from 6.8 at the beginning of the experiment to 8.4 at the end while with *Ch. globosum* the pH value remains at approximately 6.8 throughout the period of incubation. It is well known that pH value is a limiting factor in the growth of micro-organisms. After the sixth day the organism apparently adjusted itself to its new environment and proceeded at a more or less uniformly decreasing rate of growth. When the amount of carbon dioxide evolved since the beginning of the experiment is plotted against days of incubation (fig. 13, *D*) the curve shows a definite lag phase. The lag phase is followed by a very uniform rate of carbon dioxide accumulation. The curve from the fourth day to the end of the experiment is practically a straight line.

During the 15-day incubation period *Ch. globosum* produced 8.9 milliequivalents of carbon dioxide and *S. cytophaga*, 6.3 (fig. 13). The rate of carbon dioxide evolution is much more rapid for the former than for the latter organism during the first or lag phase of growth. For example, during the first 4 days of incubation four times as much carbon dioxide had been evolved by the fungus as by the bacterium. Even during the logarithmic growth phase the chaetomium showed a faster rate of carbon dioxide evolution than the spirochaete.

Apparently there is no significant difference in the growth of the chaetomium and the spirochaete when the evolved carbon dioxide is removed as rapidly as it is liberated and when it is not removed. There was no significant difference between the mean loss in weight of the strips used for the carbon dioxide tests and the mean loss when squares of the fabric were incubated for 15 days under the conditions described for all the experiments except the carbon dioxide determinations.

DISCUSSION

The surface of the fabric was completely obscured by the rough, dark grayish-green perithecia of *Chaetomium globosum* while the weave of the cloth was still apparent through the yellow gelatinous accumulation caused by the growth of *Spirochaeta cytophaga*. The hyphae of the fungus were visible on and in the individual fibers,

when they were stained by the differential staining method and examined under a magnification of 100. With the other organism the outlines of what were assumed to be the cells of the bacterium could be seen on the fibers when the same method of staining and a magnification of 900 were used. The bacterium seemed to attack the fiber from the outside while the hyphae of the fungus also penetrated the fibers.

The physical analyses show that in general the breaking strength, weight, and thickness of the fabric and staple length of the fibers decrease progressively as incubation with the organisms continued. After 15 days' incubation the fabric inoculated with *Ch. globosum* lost practically all of its breaking strength, 19 percent of its weight, and approximately 9 percent of its thickness. The staple length of the majority of the fibers was reduced to less than three-sixteenths of an inch. Determinations for staple length could not be made on the 15-day samples because the fibers were too short. For *S. cytophaga* losses of approximately 78 percent in breaking strength, 12 in weight, and 7 in thickness are obtained. The staple length of the fibers after incubation with the bacterium for 15 days was about ten-sixteenths of an inch.

The chemical analyses also gave results that show definite measurable changes. At the end of 15 days the fluidity of the samples inoculated with the chaetomium increased 7 percent; the copper number, 76; the methylene blue absorption, 59; the moisture, 10; and the ash, 116 percent. During this period 9 milliequivalents of carbon dioxide were formed per gram of dry fabric. After 15 days' incubation with the spirochaete fluidity increased 6 percent; methylene blue absorption, 41; moisture, 9; and ash, 77 percent. There was a 2-percent decrease in copper number and 6 milliequivalents of carbon dioxide were formed.

The evolution of carbon dioxide is believed to be a good measure of the relative rates of growth of the two organisms. Although *S. cytophaga* produced less carbon dioxide than *Ch. globosum* it might be argued that it formed larger amounts of intermediate products. The results obtained by the physical and chemical analyses, however, do not indicate that such was the case.

All the physical and all the chemical changes produced by the chaetomium were larger than those produced by the spirochaete during the same length of time. This disparity may be caused by the difference in the rate of growth during the lag phase, the difference in the enzyme activity of the two micro-organisms, the manner in which the microbiological attack occurred, or by a change in the pH value of the agar. The pH value of the agar on which *Ch. globosum* grew remained approximately neutral while that of the agar after incubation with *S. cytophaga* changed from 6.8 to 8.4. This increase in pH value may be due to the utilization by the bacterium of a greater amount of acid than basic radicals.

During the course of this study an attempt was made to extract the enzymes from the fabric incubated for 15 days with the chaetomium and to learn whether this extract would deteriorate sterilized fabric. The results are negative, probably due to the failure to obtain the enzymes in the extract. This line of investigation should be continued.

The difference between the action of the chaetomium and the spirochaete is shown not only by the difference in the magnitude of the changes in the various physical and chemical properties but also by the copper-number results. Degradation of the cellulose by the fungus caused a gradual increase in the copper number, while the analysis of the fabric attacked by the bacterium showed that the copper number decreased for the first 6 days of incubation and then increased to the end of the experiment. This would seem to indicate that in the latter case the first materials utilized were more or less degraded celluloses with an aldehydic or ketonic structure.

The chemical methods of analyses do not indicate that there was any appreciable accumulation of degradation products of cellulose. It seems likely that the cellulose-degradation products were consumed before more cellulose was attacked, since relatively so little chemical change accompanied the considerable loss in breaking strength, weight, and thickness. No attempt was made in this investigation to determine any water-soluble decomposition products. The relatively small increases in all the chemical tests show that the action of these micro-organisms on cotton was quite different from that which causes a degradation by means of acids and oxidizing agents.

Logistic or growth curves were found to fit the breaking-strength data for both organisms and the copper-number results for *Ch. globosum*. Second- or third-degree polynomial curves, which other investigators have used to fit growth data, were found for weight, thickness, fluidity, methylene blue absorption, moisture, and ash. Most of these curves have their greatest rate of change a few days after the beginning of the period of incubation. This increase is followed by a decrease in rate. In a number of cases the rate finally became negative. Since so many of the changes in properties are expressed by similar equations, it seems possible that these changes are interrelated.

SUMMARY AND CONCLUSIONS

A bleached, desized 14-ounce cotton duck was sterilized, inoculated with *Chaetomium globosum* and with *Spirochaeta cytophaga*, two distinct types of cellulose-decomposing organisms, and then incubated on a mineral-salts agar. Samples of the fabric were removed at various intervals of time up to and including 15 days for the fungus and 18 days for the bacterium, and then tested physically and chemically.

Both types of organisms caused a decrease in warp- and filling-breaking strengths, in weight, and in thickness of the fabric. Staple-length determinations further indicated that the strength was rapidly destroyed, and that even with extreme care during preparation of the samples considerable breakage of the fibers resulted. Toward the end of the period of incubation the rate of loss of weight and thickness of the fabric treated with the fungus decreased whereas that of the bacterium increased. There was no significant difference in loss of weight when the carbon dioxide produced was removed as it was formed and when it was allowed to accumulate.

The penetration of the hyphae into the fibers was shown by a differential staining method developed during this investigation.

Fluidity, methylene blue absorption, moisture content, and ash content increased during incubation. Copper numbers of the fabric treated with *Ch. globosum* became progressively greater while that of the material incubated with *S. cytophaga* at first decreased and then increased. Considering the almost complete loss in strength the changes in fluidity, copper number, and methylene blue absorption are much lower than those reported when the breaking strength of the fabric is reduced to the same extent by acids or oxidizing agents. The large increase in ash content indicates an increased absorptive capacity for mineral salts.

A method for estimating the evolution of carbon dioxide was modified in such a way as to make it applicable to fabrics. This method which was used to measure the rate of growth of the organisms showed a period of decreased carbon dioxide evolution for the chaetomium during perithecium and spore formation. A sharp break in the rate of carbon dioxide evolution for the spirochaete after the sixth day may be attributed either to the formation of alkaline decomposition products or to the utilization of more acid than basic radicals from the agar medium.

Under the conditions of the experiment *Ch. globosum* deteriorated the fabric more rapidly and more completely than did *S. cytophaga*. During incubation with the spirochaete the pH value of the agar changed from 6.8 to 8.4 while that of the agar on which the chaetomium had grown remained at approximately 6.8. This change in pH value is suggested as a limiting factor in the growth of *S. cytophaga*.

In a study of the mathematical relationships of the data it was found that logistic growth curves fitted all the breaking-strength values as well as the copper-number results from *Ch. globosum*. Second- and third-degree polynomial equations were found for weight, thickness, fluidity, methylene blue absorption, ash, and moisture. It is felt that these greatly facilitated certain interpretations of the data.

The results presented in this paper were obtained when the micro-organisms were provided with more or less optimum conditions. It is realized that comparable results might not be obtained when cotton fiber and fabric is deteriorated under natural conditions.

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