R47-12

Fundamental Aspects of the Prevention of the Microbiological Degradation of Cotton Textiles*

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Abstract

As a result of intensive work on the problem by the Quartermaster Corps, the N.D.R.C. and collaborating institutions, over 10,000 cultures of microorganisms have been isolated from deteriorated cotton fabrics. Of these, about 200 cultures have shown to possess cellulolytic ability. It is doubtful, however, whether all these are of considerable importance under field conditions. In attacking the cotton fiber the fungal hypha penetrates the fiber wall into the lumen where it proliferates and digests the cotton fiber outwards. Bacteria, on the other hand, adhere to the outer surface and pit their way inwards. In both cases, the attack appears to be a highly localized affair with degradation occurring only at the point of the fiber in immediate contact with the microorganism.

The organisms secrete cellulose-digesting enzymes. So far, these have been classified into 2 classes, viz.: (a) cellulase, which converts cellulose into cellobiose and (b) cellobiase, which converts cellobiose into glucose. Cell-free preparations of these cellulose-degrading enzymes have been made and their properties extensively studied.

There are 4 general lines of approach to the development of preventive methods, viz.: (1) physical prevention of organism from making contact with the cellulose molecule, as illustrated by the resistance of resin-impregnated cloth, (2) cell toxicants, as exemplified by fungicides, (3) specific enzyme inhibitors which exist today as a theoretical possibility, and (4) chemical modification of the cellulose molecule, which appears to be very promising for future exploitation.

Introduction

THE significance of the microbiological degradation of cotton textiles was first impressed upon the military mind during the early days of the South Pacific Campaign of the last war. Coupled with warm temperatures, the prevailing dampness of the Tropics stimulated microorganisms to degrade tentage and clothing at a very rapid rate. Typical reports of this wide damage is a British one estimating that the loss attributable to microbiological degradation in the Ordnance Depots in India for the last three months of 1944 alone amounted to about a quarter of a million dollars (*1*). During those arduous days of war the financial losses were even overshadowed by the tactical and morale setbacks. Faced with the urgency for a solution of the problem, the Quartermaster Corps began concerted efforts towards the development of microbiologically-resistant cotton fabrics. The necessity for an immediate stop-gap measure, at least, left no alternative at the outset but to work with what we knew and had. This meant that efforts were considered only in relation to the availability of large scale commercial processing. The resulting program of research was based primarily on the empirical development of fungicides, which could be applied to the cloth in the piece by standard textile machinery.

While this program resulted in a temporary relief, it soon was recognized that progress was being hampered by the lack of information concerning even the most elementary and basic aspects of the question. In cognizance of this state, an active and extensive program was initiated on the elucidation of the fundamental facts of the problem. I should like to survey the highlights of such investigations and their relation to the development of sound preventive methods. Most of the data to be presented represent work that has not been published. It is expected, however, that a monograph on the subject will be put out by the Quartermaster Corps in the not too distant future.

Essentially, the program was divided into 3 phases:

(1) What are the identity and physiology of the organisms concerned?
(2) How do these organisms bring about the degradation?
(3) Based on this fundamental information, what preventive methods can be developed?

Cellulolytic Microorganisms

As far as the organisms are concerned, about 70 species of the 10,000 cultures of fungi, bacteria, and Actinomyces isolated by our laboratories and collaborators from deteriorated fabrics have been shown to be capable of degrading cellulose. These organisms vary greatly in their ability to degrade cellulose. The fungus, *Myrothecium verrucaria*, and the bacterium, *Sporocytophaga myxococcoides*, are among the most active, being able to reduce the tensile strength of cotton cloth to zero within a week under optimum conditions. Other species like *Cladosporium herbarum* cause a reduction in tensile strength of only about 25 per cent in 2 weeks.

Although these organisms can attack cellulose in an otherwise inorganic medium, there are others which require the addition of organic substances. *A. niger*, J745, is an example of one requiring the addition of yeast extract; *Memnoniella echinata* requires biotin.

Besides this differential nutritional requirement among different microorganisms, their cellulolytic activity is also greatly influenced by the environment. Temperature and pH are important considerations, which have been studied extensively in the Quartermaster laboratories. The pH of the medium influences the rate of cellulolytic action to such a significant extent that for comparable results either in experimental work or specification tests, the various factors such as temperature, pH of the medium, and nutritional levels should be controlled rather carefully.

However, the single fact of the classification in the laboratory of the organism as a "strong" cellulose destroyer under specified pH, temperature, and nutrient conditions, does not imply that it is important in the deterioration of fabrics in the field. There are as yet unknown natural operating factors which control their activity in the field. Thus, in all of the field work conducted by the Quartermaster Corps, there is no evidence as yet to indicate that *Myrothecium verrucaria*, which is perhaps the most strongly cellulolytic in laboratory tests, is of great significance in the field. There have been few instances where this microorganism has been observed fruiting on cotton fabrics in the field. The dominant fungi in the field include *Memnoniella echinata* and *Chaetomium globosum*. Under wet field conditions it is highly probable that bacteria, such as the cytophagas, play a significant role in the ultimate breakdown of cloth.

* Presented before the Fundamental Research Session, Silver Jubilee Convention, Boston, Mass., December 13, 1946.

Mode of Histological Attack on Cotton Fibers

Preliminary work has been done by some authors (*2*) on the histological penetration of cotton fibers by microorganisms. In the case of the fungus, it appears as if the hypha penetrates the wall of the fiber, advancing into the lumen. There the fungus proliferates vigorously degrading the fiber from the inside outwards. In the case of bacterial attack, the organism adheres to the surface of the fiber and pits its way inwards. There is rarely an invasion of the lumen by the bacterium until nearly complete degradation of the fiber sets in (*3*). Although these organisms differ in their gross method of attack on the fiber, there is similarity in their highly localized action. The cellulose seems to be degraded only at the point of immediate contact of the organism with the fiber. There appears to be little action at a distance away from this point of contact.

Resin-impregnation as a Preventive Method

This fact offers a premise on which to develop a microbiologically-resistant fabric, viz.: an interruption of the direct contact between the microorganisms and the fiber by an inert physical barrier. This can be accomplished by means of certain resin-impregnations. Small scale laboratory trials have shown this method to be capable of imparting a high degree of microbiological resistance to the cloth.

Cell Toxicants as a Preventive Method

The second rather obvious preventive method, of course, is the use of cell toxicants. Many theories are being advanced to explain the action of fungicides, but they are still in a state of generality. There is the theory of the inhibition of metal catalysis, for example. Zentmyer (*4*) invoked this theory to explain the action of 8-hydroxyquinoline on *Fusarium oxyporum*. This organism is known to require zinc for growth. He found that if the culture medium was enriched with zinc the fungus will grow in the presence of the fungicide. The theory presumes that the zinc is required by the fungus in the form of a free ion and that the hydroxyquinoline forms a complex ion with the small amounts of zinc in the medium. This results in the zinc being made unavailable to the microorganism. But in the presence of extra amounts of zinc, there will be enough free ionic zinc left over after all of the hydroxyquinoline have been tied up.

Other theories of fungicide action involve the disruption of vital enzymatic processes in the protoplasm of the microorganism (*5*). Enzymes consist of 2 parts as a rule, viz.: a protein part and a prosthetic group. The prosthetic group is usually a simple dissociable compound, which is the functional end of the complex. Enzymatic action can occur if the prosthetic group is linked onto the protein part. Certain types of cell toxicants are said to interfere with the normal functioning of these enzymes by denaturing the protein part. Other cell toxicants may interfere with the combination of the prosthetic group with the protein part of the enzyme. Still other toxicants combine with the prosthetic group on the protein in such a strong chemical union that the prosthetic group cannot participate in its normal reactions.

All of these cases result in the elimination of one of the vital agents for life processes in the cell, thereby bringing on death.

As far as tolerances to given cell toxicants are concerned, organisms behave with great variation. The explanation is not entirely clear. It may lay in the differential permeability of the cell walls of various species to the compound. A fungus has been found which grows in very concentrated copper solutions, presumably because the cell wall is impermeable to copper (*6*). Or the reason may lay in a differential capacity for detoxication by the protoplasm of respective microorganisms. In any case, these considerations make it improbable that compounds can be found with equal toxicity for all organisms. If we wish to find a generally effective fungistatic agent for preventing the microbiological breakdown of cotton cloth therefore, we have to work from a different angle based on a knowledge of the mechanism of degradation of cellulose.

Specific Enzyme Inhibitor as Preventive Method

When we begin to investigate more in detail as to what happens to the cellulose micelles and cellulose molecules during the microbiological degradation of the cotton fiber, we begin treading on strange grounds. Available hypotheses concerning this phase of the work need much experimental support before they can be accepted. For example, the suggestion has been made that microorganisms preferentially remove the amorphous cellulose. It has been claimed (*7*) that this initial removal of amorphous cellulose, which were presumed to be the elastic cohesive medium between micelles to form fiber units, was the factor giving rise to a decline in tensile strength of the cloth.

As equally preliminary is our experimental knowledge of the biochemical reactions undergone by the cellulose molecules themselves as they are being used by the microorganism as a source of carbon.

We know from our chemical knowledge of the macromolecular nature and insolubility of cellulose that cellulose molecules as such cannot enter the microorganism. Rather, it must first be broken down to yield water soluble products, which subsequently diffuse into the cell. We are particularly interested in the chain of chemical reactions involved in the transformation of the macromolecular compound into water soluble substances. Two types of mechanisms have been proposed, viz.: an oxidative and a hydrolytic one.

The oxidative theory (*8*) assumes that the 6-hydroxy group is converted into the 6-aldehyde, thence to the 6-carboxylic compound. The last is subsequently cleaved into water soluble compounds. Preliminary studies have shown that 6-carboxy-cellulose, as well as cellulose-2,3-dialdehyde, were both resistant to *Myrothecium verrucaria*. These observations, therefore, do not support the oxidative theory.

A hydrolytic route had been previously proposed (*9*), which has been adopted as a working hypothesis by most people in the field. In any case, the conventional transformation for the microbiological degradation of cellulose has stipulated the conversion of cellulose to cellobiose by the enzyme cellulase and the breakdown of cellobiose to glucose by the enzyme cellobiase. In arresting the development of bacteria growing on cellulose at different stages, it is possible to cause cellobiose and glucose to accumulate in the medium, thereby suggesting that cellobiose and glucose are actual products of the enzymatic cellulolytic process. Several people have since confirmed the accumulation of reducing substances (*10*, *11* and *12*). Cell-free preparations capable of degrading cellulose have been made. The properties of these enzyme preparations and their relationship to the microbiological cellulolytic process is being studied in Quartermaster laboratories. There seems to be considerable evidence that this hydrolytic equation is probably the actual chain of reactions. Theoretically, we should be able to find inhibitors specific for the cellulase reaction itself. If the first reaction of the chain is stopped, we will not have to concern ourselves with the rest. Preliminary studies indicate some possibilities in this approach. Since it is reasonable to assume that the same or similar mechansm is used by different microorganisms for the degradation of cellulose, an inhibitor developed on this basis will probably be effective against most or all species of cellulolytic microorganisms. Of course, this basis has not been developed sufficiently beyond the theoretical and laboratory stages to make it industrially exploitable today. However, it should be borne in mind in the future development of fungicides.

Chemical Modification of Cellulose Molecules as a Preventive Method

Further considerations of the biochemical mechanism of the cellulolytic process will lead us to the theoretical basis for the fourth preventive method for the microbiological degradation of cotton fabrics. This involves the chemical modification of the cellulose molecules on the surface of the fibers into resistant derivatives. An example of this is the acetylated cloth first tried by Thaysen (*13*) in England, subsequently being carried further by the Southern Regional Research Laboratory in this country. If a substituent is placed at the proper hydroxyl group or groups on the cellulose molecule, it will eliminate susceptibility of the cloth to microorganisms.

This may be due to either one or both of the following reasons:

(1) The group on the cellulose molecule which normally is the point of attack by the microorganisms may be changed so that the requisite biochemical reaction can no longer take place. This results in the non-digestibility of the cellulose derivatives on the fiber surface.

(2) The substituent introduced may change the steric pattern of the cellulosic substrate so as to interfere with the action of the enzymes secreted by the microorganism. In the light of present day theories of enzymatic reactions, the molecular shape of the enzyme secreted by the organism must fit the shape of the cellulose molecule before the breakdown of cellulose can take place. If a chemical substituent is introduced into the cellulose molecule, the steric pattern of the substrate is modified; the resulting cellulose derivative is no longer sterically compatible with the enzyme secreted by the organism; the subsequent enzymatic reaction cannot occur; and the derivative is thereby not susceptible to the microorganism. Since the surface of the fiber is coated with this resistant derivative and since, as we have said before, the action of microorganisms is highly localized, the fiber coated with layers of resistant derivatives will be immune from attack.

Theoretically, as long as we have a firm chemical substituent on the cellulose molecules it will impart resistance to microorganisms. Beyond that, the nature of the substituent does not influence the resistance much. Cooperative experimentation between industry and Quartermaster laboratories has resulted in dozens of different modified cloths, which are resistant to microorganisms. With a bit of extrapolative reasoning, one should be able to pick substituents which impart additional desirable properties other than microbiological resistance to the cloth. Groups with water-repellency qualities should impart both water-repellency as well as microbiological resistance to the cloth if they can be chemically bonded to the cellulose molecule. Through the same process, flame-proofness can be coupled with microbiological resistance by means of a judicious selection of reagents.*

Because of the relatively recent development of this concept of chemical modification, however, we did not have opportunities for sufficient comparisons between the relative permanency of the microbiological resistance as imparted by this method as compared against that obtained through fungicidal impregnations. Theoretical considerations, however, favor the chemical modification method. As discussed previously, the cell toxicants prevent the breakdown of cloth by killing the invading organism. This toxic action takes place through a diffusion of the compound into the cell of the organism, where its action is affected. This necessarily implies a solubilizing of the compound, however slight. In the development of fungicides, therefore, efforts must be directed among other things towards the compound which is not so soluble that it will be leached off by rain easily but yet possesses a solubility sufficient for the exertion of its toxicity upon the cell protoplasm. The chemical modification method, however, operates on a different principle. It is based on the inability of the enzymes secreted by the microorganism to attack the substrate outside of the organism. There is no requirement for a solubility of the cellulose derivatives for its preventive action. As a matter of fact, the more insoluble the derivative the better it is for the purpose. Furthermore, fungicides are usually physically adsorbed on the cloth while chemical substituents in a chemically-modified fabric are integral parts of the fiber surface.

Besides this potential permanence there are other advantages in the use of the chemical modification method, which can be deduced from our previous discussion on the specificity of cell toxicants. We showed that when the method of controlling the action of microorganism is dependent upon the diffusion of a cell toxicant into its protoplasm there is considerable play for differential tolerances by various organisms. We also showed that when the prevention is outside of the cell based on a control of the action of the extracellular enzymes secreted by the microorganism there is great hope for a general control of the action of all cellulolytic microorganisms. Since the chemical modification of cellulose molecules is based on the prevention of extracellular enzymatic action it ought to be effective against most or all cellulolytic microorganisms.

Our fundamental research and small scale developmental work indicated that the method of the chemical modification of the cellulose molecules on the surface of the fiber has very considerable possibilities. We believe that this great promise should be tracked down to its ultimate practical uses, both through further fundamental work to fill in the gaps in our knowledge about the mechanism of action of the microorganisms and larger scale developmental work.

* The "Ban-Flame" finish of Joseph Bancroft & Sons Co. of Wilmington, Delaware, is one of the first developments along this line. It is understood that patents are pending thereon and that licenses may be obtained.

Conclusion

Our preceding discussion suggests that there is sufficient basic information on the subject for us to proceed on the practical solution with surefootedness. We believe that vast practical strides can be made in the near future through proper cooperative efforts between the institutions represented by you here at this meeting and the Quartermaster laboratories.

References

1. Weston, W. H., Proceedings of the Conference on Quartermaster Textile Research. The Inter-society Council for Textile Research, p. 29, October 25, 1945.
2. Bright, T. B., J. Text. Inst. *17*:T396-404 (1926).
3. Stanier, R. Y., Bact. Rev. *6*:143-196 (1942).
4. Zentmyer, G. A., Science *100*:294-5 (1944).
5. Sevag, M. G., Advances in Enzymology *6*:33-121 (1946).
6. Starkey, R. L., and Waksman, S. A., Jour. Bact. *45*:509-519 (1943).
7. Royal Aircraft Establishment, Farnborough, Eng. (Castle V.) Chemistry Note, No. 770, September 20, 1945.
8. Winogradsky, S., Ann. Inst. Pasteur *43*:549 (1929).
9. Pringsheim, H., Z. Physiol. Chem. *78*:266 (1912).
10. Karrer, P., et al, Helv. Chim. Acta. *7*:144, 154 (1924); *9*:893 (1926); *11*:229 (1928).
11. Kalnins, A., Acta. Univ. Latviensis Lauksaimniecibas Fakultat (Ser. 1) *11*:221 (1930).
12. Simola, P. E., Ann. Acad. Sci. Fennicae (A) *34*:1 (1931).
13. Thaysen, A. C., and Bunker, H. J., "The Microbiology of Cellulose, Hemicellulose, Pectin and Gums," London (1927).

Discussion

Mr. Normile: Dr. Siu, is it possible, under certain conditions, that organisms may secrete acidic constituents that may attack the cellulose directly, due to the low acidity rather than the enzyme?

Dr. Siu: Highly improbable. If the pH of the medium is too low, the organism cannot grow. Usually, when the pH of a medium gets down to about 4, the fungus cannot grow. It is unlikely, therefore, that the fungus will secrete anything more acid than that, to cause any damage to cellulose.

Mr. Shaw: What about changing the structure of the molecule by mercerization and its effects on mildew resistance?

Dr. Siu: Mercerization actually increases the mildew action on the cloth. But whether the mercerization process gives you a stable chemical derivative, is questionable. If you have an unstable chemical substituent on a cellulose molecule which will revert back to the original cellulose, naturally the so-called derivative will not be resistant to microorganism.

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AMERICAN DYESTUFF REPORTER
June 16, 1947