XVIII. STUDIES OF THE BACTERIAL DECAY OF TEXTILE FIBRES¹.

I. VARIATIONS IN THE RESISTANCE OF COTTONS OF DIFFERENT ORIGINS TO DESTRUCTION BY MICROORGANISMS.

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THE swelling test, a method for the quantitative determination of the destruction of cotton fibres caused by microorganisms was described in this Journal by one of the authors in collaboration with Miss Nancy Fleming [1921]. In the paper in question attention was drawn to a number of problems arising out of the application of this method to the study of the bacterial decay of cotton. Some of these problems have since then been more carefully investigated and the results obtained are discussed in the following pages.

Before dealing with these results, however, the authors wish to draw attention to a paragraph in the paper referred to which has, it is thought, given rise to a serious misunderstanding [Denham, 1922]. In discussing the respective merits of the swelling test and the alkali solubility test for the determination of the extent of bacterial destruction of cotton it was stated that "while the swelling test (Table V) registers a steady rise in the percentage of physically damaged fibres, the alkali solubility test (Table VI) remains more or less constant until the destruction of the cotton has reached a point when it is affected chemically and may be easily detected even by the inexperienced eye." It is very much to be regretted that the wording of this paragraph left room for the interpretation that the damage observable by the swelling test was of a physical nature as distinct from a chemical damage, detectable only by the alkali solubility test. This interpretation was not intended and a closer examination of the two tables, V and VI of the paper to which reference is made, will show that no such differentiation is possible. Whether measured by the swelling test or by the alkali solubility test, the nature of the damage is obviously the same. And since the damage must be regarded as a chemical change of the cellulose by the time it is observable

¹ Though this paper is to be regarded as a continuation of two previous papers appearing in the *Biochemical Journal* under the title of "Deterioration of Cotton on Wet Storage" the title for various reasons has been altered as above.

by the alkali solubility test, it must have been of this nature in its early stages, that is, when it was only observable by the swelling test. It should not be assumed therefore, as Denham [1922] has done, that fibres in which the cuticle has been mechanically damaged, will behave in the same way as bacterially damaged fibres, when subjected to the swelling test. In such mechanically damaged fibres the cellulose itself generally has not undergone any change and should therefore behave like that of normal cotton fibres on swelling. That it actually does this has been repeatedly confirmed by the authors in the course of their investigations. It has further been suggested [Denham, 1922] that the swelling test would give fallacious results in cases of cotton fibres which had been attacked by microorganisms deposited in the central canal. From what has been said it will be understood, that, if an attack on the cellulose has been made by microorganisms from the central canal, the cellulose must have been changed in the same way as it would have been had the attack proceeded from without. And in such cases the damaged cellulose should respond in the usual way to the swelling treatment. It has not, unfortunately, been possible to examine fibres which had been attacked from within the central canal (such cases appear to be extraordinarily rare), but where microorganisms have been observed both within the central canal, and on the surface of the fibre, the swelling test has never been observed to fail.

In the publication by Fleming and Thaysen already referred to [1921] it was mentioned that the susceptibility of raw cottons to destruction by microorganisms appears to vary with the country of their origin. Thus American cottons were found to show greater resistance to attack than Egyptian, and these again greater than Indian cottons. This observation has now been confirmed on a total of 15 different American, 15 Egyptian, 35 Indian and 8 Indo-American cottons and the curves shown in Fig. 1 represent the approximate rates of destruction of the three mentioned types as measured by the swelling test. It is very noteworthy that the rate of destruction of each type is practically constant, so much so in fact, that on several occasions it has been possible to identify raw cottons by means of their rate of destruction by microorganisms.

It will be understood that both the constancy of the rate of destruction, and the variation in the susceptibility to attack are observations of considerable theoretical and practical importance, pointing as they do either to a difference in the chemical or physical nature of the fibres of the various types of raw cotton, or to the presence of inhibitory substances in the more resistant forms of fibres. Further, they open up possibilities of improving existing strains of cotton as regards their resistance to destruction by bacteria by the introduction of selective breeding in the propagation of seed. The importance of such work will be appreciated when it is mentioned that the U.S. Department of Agriculture estimate that the annual losses suffered by that country, and due to the bacterial destruction of raw cotton amount to about 70 million dollars.

In considering the question of the presence of inhibitory substances it is convenient to take into account the importance of the presence or absence of food substances, since it may well be that what appears to be an inhibitory action is nothing more than a lack of essential foods. The connection between the presence of food substances and the rate of deterioration of cotton fibres was determined in an experiment which is quoted in Table I. In this experiment an American, an Egyptian and an Indian cotton were wetted with water containing 0.1 % each of peptone and potassium hydrogen phosphate.

Fig. 1.

Table I.

Sample		Percentage of decomposed fibres found in the cottons after incubation at room temperature, 16°, for							
		0	6	12	18	24	30	37	42 days
American	wetted with food solution	0	1	1	1	2	5	15	21
Egyptian	" "	0	1	1	1	4	16	18	35
Indian	" "	0	0	3	18	26	31	34	39
American sample = "American Lint."		Egyptian sample = "Sakel Combed Sliver."							
		Indian sample = "Auranga."							

Comparing the figures in Table I with the curves of Fig. 1, it will be seen that the rate of destruction is very little affected by additions of food. Quite a different behaviour, however, is shown by samples of American cotton grown in India. The rate of destruction of two samples of this type was reported on by Fleming and Thaysen in the publication already quoted, and was shown to be practically the same as that of typical American cottons. It has been possible to confirm this on eight further samples of Indo-American cottons,

which were wetted with tap water or distilled water. However, where food substances such as a mixture of peptone and K_2HPO_4 were added, the rate of deterioration of the Indo-American samples increased very considerably and became practically as high as that of ordinary Indian cottons. This is shown in Table II, where the results obtained with a Cawnpore American cotton are tabulated.

Table II.

Sample of Cawnpore American wetted with	Percentage of decomposed fibres in the cotton after incubation at room temperature, 16° for						
	0	6	12	18	24	30	37
Distilled water	0	0	0	2	1	1	1
Tap water	0	0	1	1	3	2	1
Food solution ¹	0	—	5	7	8	34	35

¹ Containing peptone 0.1 % and K_2HPO_4 0.1 %.

Here, in the case of the Cawnpore cotton, there is no doubt that the addition of food caused an increased deterioration. Similar results have been obtained with other strains of Indo-American cottons, and the resistance of these strains may therefore well be ascribed to the absence from the fibres of sufficient food substances for the development of microorganisms. But it is different with the true American and Egyptian cottons. It has already been shown that the addition of food is without appreciable effect on their rate of destruction, and at one time it was thought that the fibres of these cottons contained substances inhibitory to the growth of bacteria. The experiments which were carried out to prove this were not very conclusive. On the contrary they appeared to indicate that the resistance of the fibres was increased rather than diminished by the treatment to which the samples were subjected for the removal of inhibitory substances. This was the case for instance in the experiment quoted in Table III, in which a sample of Egyptian cotton (Sakel Combed Sliver) was boiled for half-an-hour in distilled water before exposure to the destructive microorganisms, while another sample of the same cotton was treated for three hours with 3 % NaOH at 45 lbs. pressure before infecting. In both of these cases the rate of destruction

Table III.

Sample	Percentage of damaged fibres found in the cotton after exposure to cellulose- destroying microorganisms at 16° for					
	0	6	12	18	25	30 days
A	0	0	6	24	27	33
B	0	0	6	16	21	26
C	0	0	6	13	12	14

- A. Sample of Sakel Combed Sliver, damped with distilled water in which food was dissolved (peptone and K_2HPO_4 0.1 % of each), and then infected with cellulose-decomposing microorganisms.
- B. Sample of Sakel Combed Sliver, boiled for 30 minutes in distilled water at atmospheric pressure, then damped with distilled water containing food as above and infected.
- C. Sample of Sakel Combed Sliver, heated with 3 % NaOH at 45 lbs. pressure, then mixed with the same food substances as above and inoculated with the cellulose-destroying microorganisms.

was slower than that of the original raw cotton, though food substances were added to all samples. The above treatment of boiling with water, and with alkali under pressure, was chosen in order to remove inorganic water-soluble compounds (boiling with water) and organic compounds of a fatty or waxy nature (treatment with 3 % NaOH) which might be thought to be capable of preventing the development of microorganisms.

That the higher resistance of the American and Egyptian cottons might be due to the presence of a thicker or more impenetrable cuticle has also been considered. This assumption, however, does not appear justified since (Table III) the treatment with caustic soda at 45 lbs. pressure to which one sample was subjected retarded rather than increased the destruction. Besides, had the presence of a particularly resistant cuticle been responsible for the resistance, it would be expected that, once this obstacle was overcome, the breakdown of the cellulose itself would proceed as fast as in the case of Indian cottons. That this is not the case may be seen in Fig. 1. The greater resistance of the American and Egyptian cottons maintains itself throughout the attack. It appears, therefore, that the cause of the resistance of these cottons is a property of the cellulose itself. Whether it is due to a physical or to a chemical property of the cellulose cannot at present be stated.

One further conclusion may be drawn from the experiments already discussed. The sample of Cawnpore-American cotton which was used for the experiment in Table III and which was found to have lost its resistance in the presence of food substances was a strain propagated by Burt [1921] in pure culture, which had not been allowed to interbreed with pure Indian strains. Its loss of resistance, therefore, must have been caused by climatic or soil conditions.

The value of various food substances for the rate of deterioration of Indo-American cottons has been investigated at some length; the results of the experiments are tabulated in Table IV.

Table IV.

Sample of Cawnpore cotton containing	Percentage of damaged fibres found in the cotton after exposure to cellulose-destroying microorganisms at 16° for							
	9	12	18	24	30	37	42	48 days
¹ Peptone 0.1 %	0	0	1	4	6	9	11	23
¹ Sodium nitrate 0.1 %	0	0	1	3	5	7	7	10
¹ Pot. hyd. phos. 0.1 %	0	0	1	2	3	3	6	7
¹ Peptone 0.1 %, pot. hyd. phos. 0.1 %	0	5	?	8	34	35	44	—
Control without food	0	0	1	1	1	1	1	5

¹ Calculated on the moisture content of the cotton.

It is of interest to note that nitrogen, and particularly organic nitrogen, increases the rate of destruction of Indo-American cottons very appreciably, though not quite to the same extent as a mixture of phosphates and organic nitrogen. Under the usual storage conditions, Indo-American cottons are, therefore, as liable to deterioration as typical Indian samples, since it is

practically impossible to prevent the contamination of the cotton with traces of organic nitrogen and other food substances such as phosphates.

From the observations discussed it will have been seen that there are two distinct reasons for the resistance of certain raw cottons. It may be due either to the absence of food and particularly of organic nitrogen, or to some unknown property of the fibres. In the first case the resistant cotton might be expected to contain little organic nitrogen or at least considerably less than Indian cottons which are always found to deteriorate rapidly.

Little information is available as to the nitrogen content of raw cottons. Fargher and Withers [1922] quote figures by Schindler; these figures, however, do not include typical Indian cottons. The content of American cottons is given as 0.15 % and that of Egyptian cottons as 0.25-0.257 %. Knecht quoted by the same authors, gives the nitrogen content of an American cotton used by him as 0.208 %.

The results obtained by the authors with Indian and Indo-American cottons are given in Table V.

Table V.

Sample	Origin	Weight of cotton used for determination, g.	Per cent. of nitrogen
Gadag I	Indo-American	18	0.204
I	"	22	0.207
Old Triumph	"	22	0.167
"	"	28	0.157
Cawnpore-American	"	20	0.111
"	"	20	0.123
Indian Cotton	Pure Indian	15	0.215
No. 3 Shippur Factory	"	27	0.244
M.M.X.	"	15	0.235

With all the samples the greatest care was taken to remove fragments of leaf and other impurities which had accidentally contaminated the cotton, and the results may be taken to represent the nitrogen content of the clean fibres. Leaving out the Gadag I type (which has been cultivated for a very long time in India under conditions which almost certainly would cause it to interbreed with true Indian varieties and thereby to lose its Indo-American character), there would appear to be a distinctly lower nitrogen content in the Indo-American than in the true Indian types, particularly in the Cawnpore-American sample. The difference, however, does not seem large enough to justify the conclusion that it is a lack of nitrogen which is the sole cause of the resistance of Indo-American cottons. In interpreting the above figures it should perhaps not be forgotten that all the nitrogen found may not be nitrogen available as food. The difference in available nitrogen may possibly be considerably greater than would at first sight appear from the figures in Table V.

It has already been pointed out by the authors [1923] that the swelling test may be used for the study of the breakdown of other fibres besides cotton. Linen fibres for instance have given quite reliable results when subjected to

this treatment. In Table VI figures are tabulated recording the rate of deterioration of two samples of flax, as measured by the swelling test. The two samples had been subjected to a different retting treatment, in the one case termed "dew retted," and in the other "tank retted." Whatever the difference of these treatments, the one would seem to have left the flax much more resistant to bacterial attack than the other.

Table VI.

Sample	Percentage of decomposed fibres found in the flax after incubation at room temperature, 16°, for						
	0	6	12	18	23	30	37 days
English Dew Retted Flax	0	1	2	2	5	7	7
English Tank Retted Flax	0	1	2	7	16	21	21

As regards jute and hemp, these fibres have not been found favourable objects for study by the swelling test. The individual fibres in these cases are too interwoven to make an accurate determination of their rate of deterioration possible.

CONCLUSIONS.

Using the swelling test, it is shown that raw cottons of different origin vary in their resistance to destruction by microorganisms. This resistance is probably due to difference in the physical or chemical nature of the respective fibres.

Since the resistance disappears when resistant strains are cultivated in a country producing non-resistant types, it is concluded that the resistance is adversely affected by climatic or soil conditions.

Flax fibres appear to show differences in their behaviour to bacterial destruction similar to those exhibited by cotton.

The rate of deterioration of jute and hemp fibres cannot be determined by the swelling test.

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