was incomplete, and consequently the leaf residue, freed from solvent by suction, was again extracted by refluxing 30 min with 40 ml of a 10% solution of potassium hydroxide in methanol. The two extracts were then shaken with the petroleum naphtha to remove the carotenoids. The petroleum naphtha extracts were combined and further shaken with 90% methanol to extract free and esterified xanthophylls. After washing to remove traces of alcohol the petroleum naphtha extract was dried over anhydrous sodium sulphate.

The dried petroleum naphtha extract was chromatographed on a column consisting of equal parts by weight of Hyflo Super Cel and magnesia (No. 2641 Westvaco). Two prominent zones separated; the lower, less strongly adsorbed, was removed by eluting with a 4% solution (by vol) of acetone in the petroleum naphtha. The upper strongly adsorbed zone required an 8% solution of acetone in the petroleum naphtha for elution.

The pigment in the lower zone subsequently was shown by spectrum analysis to be β-carotene. The washings of the upper zone were evaporated to dryness *in vacuo* and taken up in the petroleum naphtha. This solution showed absorption maxima in the petroleum naphtha at 4,600 A, 4,900 A, and 5,340 A, with a minimum at 4,800 A. The absorption maxima and minimum of this pigment, together with its behavior on the adsorbent column, indicated that it was identical with γ-carotene.

The total carotene content of the infected leaves was 83.2 mg/100 g of dry material, of which 28.7 mg, or 34.5%, was the γ-carotene isomer. The uninfected leaves had 32.6 mg of β-carotene/100 g of dry material. No γ-carotene was detected in the normal leaves. Moisture content of the normal leaves was 62.1% when harvested, and infected leaves contained 68.7%.

γ-Carotene is relatively rare in plants, constituting about 0.1% of the total carotene extracted from ordinary sources (*5*). Small amounts have been found in apricots (*6*). MacKinney (*7*) has reported that the marsh dodder (*Cuscuta salina*) is a relatively rich source. A considerable concentration has been found in the fruit of *Pyracantha augustifolia* Schnied (*8*). However, the concentration of γ-carotene in leaves of the crab apple (*Malus ioensis* Britt) infected with the pycnidial lesions of the common rust fungus (*Gymnosporangium juniperi-virginianae* Lk.) is the highest that has come to the attention of the authors.

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Cavitomic Cotton

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An increasing and now large proportion of the spinnable good grades of cotton received by the mills is observed to contain an abundance of cellulose-destroying microorganisms. Deterioration of cotton during warehouse storage has also been noted. The full significance is not known, but the implications are apparent.

Within the limits of our experience, the effects produced by these microorganisms include an effectively shorter staple caused by weakening of the fibers, an

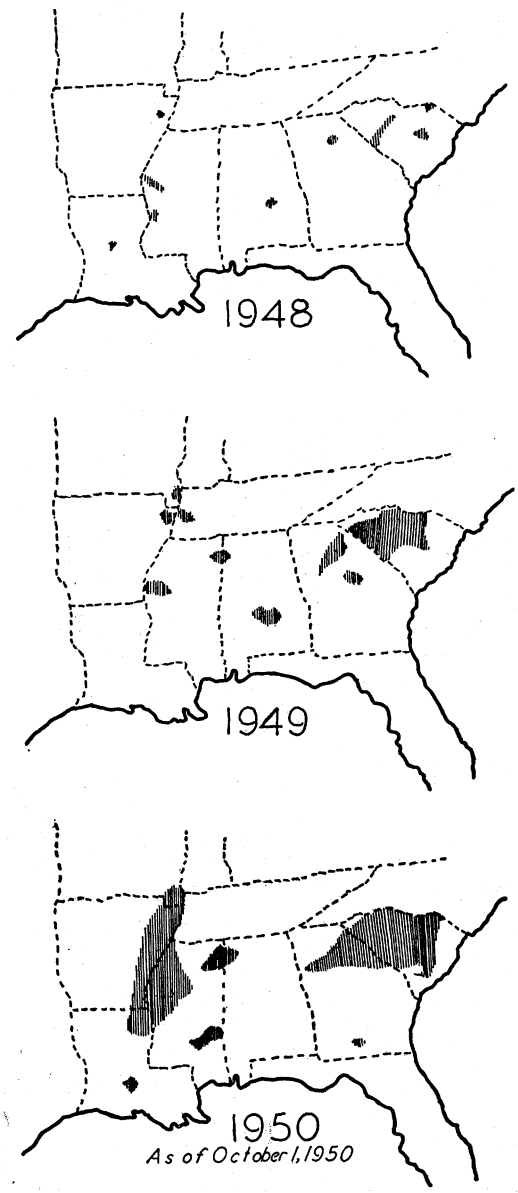

Fig. 1.

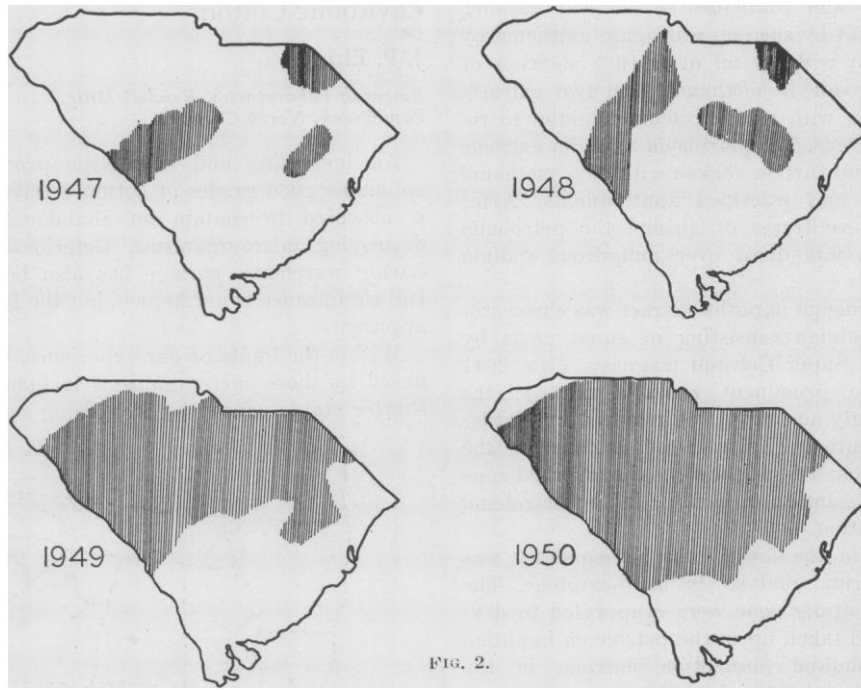

FIG. 2.

increase of "fly" and lint, the formation of dye spots in vat-dyed fabric, and an increased sensitivity to alkali. Severe loss of fabric strength after the usual alkaline kier boil and bleach has in several instances been traceable to fibers apparently damaged by microorganisms prior to the finishing process. The changes in fiber properties brought about by microorganisms are, for convenience, designated here as cavitoma.

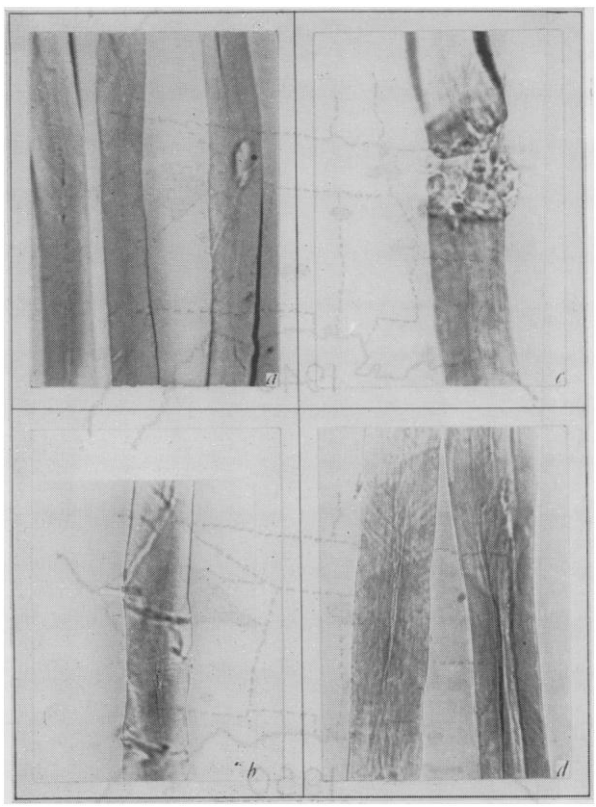

FIG. 3. Cavitomic cotton fibers (×280): *a*, "normal" fibers showing little damage; *b*, large hyphae penetrating fiber wall (note swelling); *c*, ruptured fiber; *d*, striations.

Our records show that areas from which such cottons originate are becoming large. Maps in Fig. 1 show areas from which cottons have been found to possess an abundance of these microorganisms, and Fig. 2 sketches the progress by counties of cavitoma in South Carolina as indicated by our records since 1947. These laboratories have had less occasion to examine cottons from areas west of central Texas, and hence data for these Western areas are relatively meager. Cavitoma was not observed in the Western and California cottons until last season.

This increasingly widespread prevalence of destructive microorganisms in good commercial grades of cotton and the changes in fiber properties are substantially, if not completely, unrecognized in the literature. It is for these reasons that the facts are called to the attention of readers of this journal. The information is being brought to the attention of the U. S. Department of Agriculture, within whose province corrective measures presumably lie.

The mechanism by which cavitoma spreads and is carried over from year to year has not been established. The Department of Agriculture has pointed out the unusually high incidence of tight-lock throughout South Carolina in 1949 (*1, 2*) and observed no correlation of boll rot with boll weevil infestation. Boll rot is attributed to a contamination of fiber surface from outside the boll. Bacteria and

fungi inside, as well as on the outer surface of the fiber, appear to characterize cavitoma. Cavitoma was observed in 1948 in spite of that season being considered generally excellent for growing cotton.

Cavitoma in its early stages is observed upon microscopic examination of the fibers in caustic of mercerizing strength. One observes fungi, spores, and motile bacteria external to the fiber, hyphae penetrating the fiber wall, and small or pseudo-fungi and bacteria within the lumen of the fiber. As degradation proceeds, the fibers become swollen in regions local to the infection and later throughout the fiber length. Striations of the fiber surface soon appear, revealing a crisscross fibrillar structure. As the degradation continues, the fiber becomes increasingly sensitive to the caustic and in extreme cases is virtually destroyed. The conditions seen in Figs. 3 and 4 are typical.

As deterioration of the fiber proceeds, the presence of cavitoma has also been indicated by the simultaneous existence of a very low reducing sugar content and pH values of 8–9.5 of the readily water-extractable constituents. The tests for sugar and pH have proved most satisfactory for the majority of cottons tested; occasional exceptions are noted. The possibility that fluidity measurements may prove of value in further research has been discussed by Greathouse (*3*). Whether the existence of the observed microorganisms constitutes a primary or secondary phenomenon is not known. It is assumed that the naturally existing and readily soluble carbohydrates and other nutrients provide a satisfactory medium for the initial development of the fungi and bacteria. The sugarlike constituents disappear, and then rise again to about one third their initial amount. In this latter stage the destruction of the fiber becomes strikingly apparent.

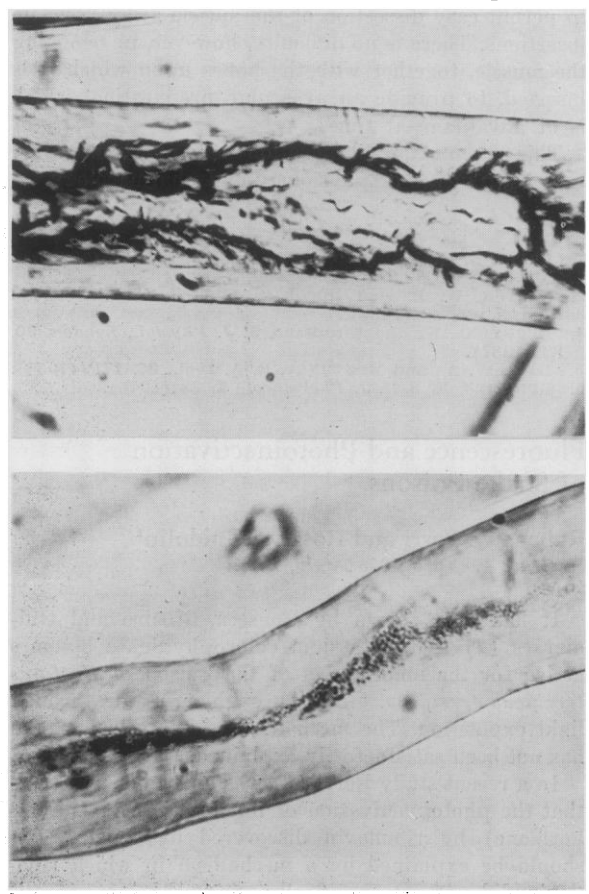

FIG. 4. Cavitomic cotton fibers stained with methyl violet (×790). Fungus or pseudo-fungus in lumen of fiber, and bacteria in lumen of fiber.

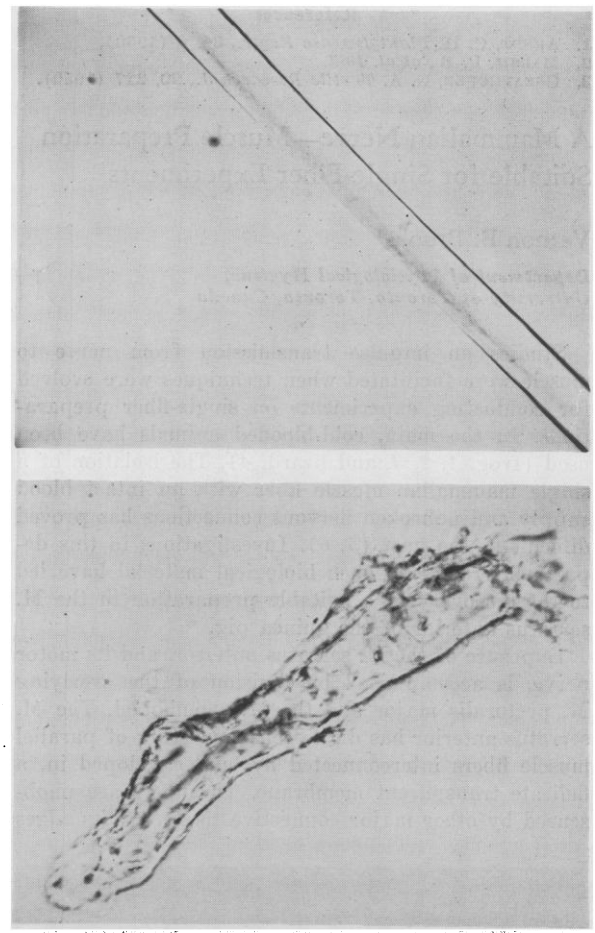

FIG. 5. Sterile and inoculated fibers after incubation (×260).

Steam-sterilized cotton fibers inoculated with a pure strain of one of the organisms from cavitomic cotton produced the result shown in Fig. 5 (*bottom*) after 1 week. Fig. 5 (*top*) shows a sterile fiber after the same incubation.

The observations described above are of concern to the manufacturer and finisher of cotton fabrics, as well as to the ginner, farmer, breeder, and plant pathologist. The scope is far beyond that of any one industrial laboratory. By presentation of this note these laboratories do not wish to circumscribe areas of research; on the contrary, it is hoped that every effort will be made by others to appraise the phenomena.

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A Mammalian Nerve—Muscle Preparation Suitable for Single-Fiber Experiments

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Studies on impulse transmission from nerve to muscle were facilitated when techniques were evolved for conducting experiments on single-fiber preparations. In the main, cold-blooded animals have been used (frog, *1, 2, 3,* and lizard, *4*). The isolation of a single mammalian muscle fiber with an intact blood supply and unbroken nervous connections has proved difficult in the past (*5, 6*). Investigations in this department requiring such biological material have led to the discovery of a suitable preparation in the M. serratus anterior of the guinea pig.

Exposure of the M. serratus anterior, and its motor nerve, is accomplished by division of the overlying M. pectoralis major and the M. rhomboidei. The M. serratus anterior has digitations consisting of parallel muscle fibers interconnected by, and enveloped in, a delicate transparent membrane. The fibers are unobscured by other major connective tissue. At its edges

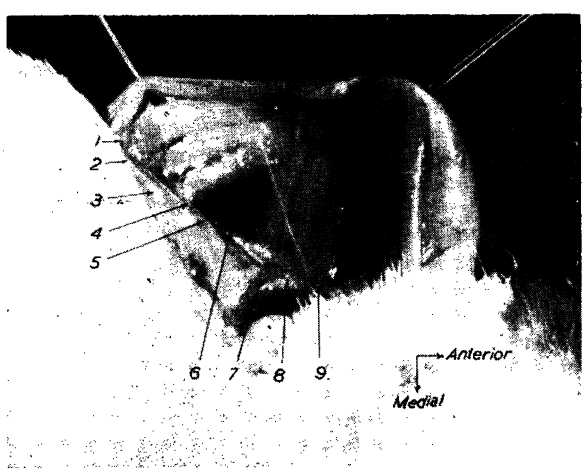

FIG. 1.

each digitation has a depth of only 1 or 2 muscle fibers. Upon laterad deflection of the scapula, the trunk and branches of the N. thoracalis longus, which furnish the motor nerve supply to the M. serratus anterior, are easily seen (Fig. 1). The end plates, or myoneural junction tissue, of the unstained living cells are readily distinguished under the microscope. A photomicrograph of such a preparation (Fig. 2)

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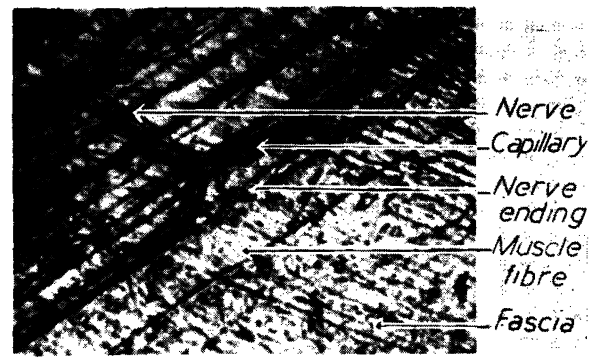

FIG. 2.

shows junction tissue between a muscle fiber and a nerve twig. The vascular supply of the muscle fibers is apparently not disturbed despite the abnormal position of the muscle. If the tissues are adequately irrigated with warm physiological saline or mineral oil the muscle fibers respond to electrical stimulation of the motor nerve for several hours after exposure. The fibers and end plates can be touched and pierced with micropipettes and microelectrodes.

The tendons of the M. serratus anterior are too short to permit easy dissection of the muscle away from its insertions. There is no difficulty, however, in removing the muscle, together with the bones upon which it is inserted, to provide an avascular preparation, which is of advantage at times.

This mammalian preparation may prove useful in various branches of physiology and pharmacology.

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Fluorescence and Photoinactivation of Snake Poisons

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It has been shown by Fonseca Ribeiro and Guimarães (*1*) that potassium chlorophyllinate becomes active for the inactivation of the poison of *Crotalus terrificus terrificus,* either through aging or through light exposition. The mechanism of this phenomenon has not been satisfactorily explained.

In a recent study Ferri (*2*) was able to demonstrate that the photoinactivation of indolacetic acid (phytohormone) by riboflavin discovered by Galston (*3*) should be explained by a mechanism in which riboflavin did not act specifically, since the same inacti-

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