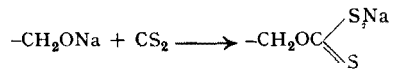
An Electron Microscopic Study of the Structure of Viscose Silk

In two previous publications[1] electron micrographs were shown of native cellulose as it is found in the cell walls of textile fibres, such as cotton, hemp and ramie, and of meristem cells as provided by the coleoptiles and roots of sprouting seeds. Viscose silk is formed from such cellulose fibres by solution and regeneration: this study has been made to see how their fine structure is altered by the chemical treatments leading to solution and to determine how much of the original structure is restored by the "regeneration" that takes place when the viscose is "regenerated" by spinning.

The large scale manufacture of viscose silk rests on a reaction worked out as long ago as 1893 by CROSS, BEVAN, and BEADLE[2]. They found that carbon disulfide transforms alkali cellulose into a water soluble compound from which cellulose itself can be recovered through the action of acids. The essentials of this well-known process are the following:—

Highly purified cellulose, so-called artificial-silk cellulose, is first "mercerized" with 17% NaOH. The treated cellulose is pressed out, shredded and allowed to stand in the air. During this "ripening" an oxidative decomposition takes place. The resulting alkali cellulose is treated with carbon disulfide vapor with the formation of viscose as a yellow water soluble substance. This is an alkali xanthogenate which, according to MEYER and MARK[3], involves the following reaction:—



When spun into the acid bath, the xanthogenate group is split off again and cellulose regained in a more desirable physical form.

It seems obvious that the original fibrillar structure of the cellulose must have been altered by these repeated chemical disintegrations. The initial rough grinding in the pulp mill brings about extensive disorientation of the finer differentiated structure of the plant cell walls. During the following treatment with alkali there is a strong swelling which must disorient the molecular array within the individual fibrils. This desirable breakup facilitates a subsequent penetration and attack by the carbon disulfide vapor. There are today two points of view as to the fine structure thus produced within the xanthogenate solution. According to one of these the solution contains long fibrous molecules. The viscosity measurements of STAUDINGER and REINECKE[4] indicate for these molecules a mean degree of polymerization corresponding to *c.* 200–400 glucose residues; such particles should have a length of from 0·1 to 0·2 micra. Their small diameters, amounting to only one glucose residue, i.e. to *c.* 7·35 Å, would make them invisible in the electron microscope.

According to the other point of view, advanced by LIESER[5], these very thin molecules are associated together in packets in the "solution". Although they are not crystalline, the term "micelle" is often applied to these packets. LIESER could demonstrate chemically that many chains were only partially sulfided; and this becomes readily understandable if such molecular associations exist to impede penetration by the reagent. Unlike the individual molecular chains postulated by the first hypothesis, these associations should be visible in the electron microscope. During injection of the xanthogenate solution into the precipitating bath these larger associations should be more or less oriented and then fixed in place by the coagulating reagent; hence even after regeneration they should be discernible as structural elements within the finished viscose fibers.

[1] K. MÜHLETHALER, Biochim. biophys. acta *3*, 15 (1949); Biochim. biophys. acta (in press).

[2] C. F. CROSS, E. T. BEVAN, and C. BEADLE, Ber. Dtsch. chem. Ges. *26*, 1090, 2520 (1893).

[3] K. H. MEYER and H. MARK, *Hochpolymere Chemie* (Leipzig 1940).

[4] H. STAUDINGER and F. REINECKE, Papierfabrikant *36*, 489 (1938).

[5] TH. LIESER, Liebigs Ann. Chem. *464*, 43 (1928); *470*, 104 (1929); *483*, 132 (1930).

Experimental

The fibers used in this investigation were samples taken from the routine production of a viscose factory. For electron microscopic examination they were shredded by 10 minutes treatment in a Waring blendor and the finest fraction from this treatment was mounted on the usual collodion-covered grids. Before study with an RCA-Type EMU electron microscope, such preparations were shadowed in the usual way with either chromium or palladium.

Results

Knowledge of the chemical reactions outlined above permits interpretation of what is seen in such preparations. Products of early steps in the process are still recognizable in finished fibers of artificial silk which therefore do not have a uniform internal texture. Even under the optical microscope and the ultramicroscope, particles are seen suspended in the otherwise structurless mass of viscose; and these have had the general appearance to be expected of undissolved remains of native cellulose fibers. The electron microscope demonstrates that these particles are far more numerous than optical observation had indicated. Such pieces of original cell wall spun into the finished viscose fiber are evident for example in Fig. 1. In the same way it is clear that the interlocking fibrillar network of Fig. 1 is a surviving bit of primary wall. Lignin, pectin and the other substances that accompanied cellulose in the original fibers seem to have been completely dissolved away with little alteration of the cellulose fibers themselves. Even the treatment with NaOH has not greatly changed them, for only the periphery of the fragment of Fig. 1 shows the effects of the chemical action. These residues of native cellulose have been seen with dimensions up to five micra, a limit doubtless set by the pore-size of the filters through which the material has been pressed.

Isolated cellulose fibrils are also found with the viscose fibers. These presumably were separated from their neighbors during the original milling. As Fig. 2 shows, they have been so grossly swelled by the alkali that they have not returned to their original dimensions on drying and evacuation. This makes it probable that the severe swelling has resulted in a break-up and disorientation of

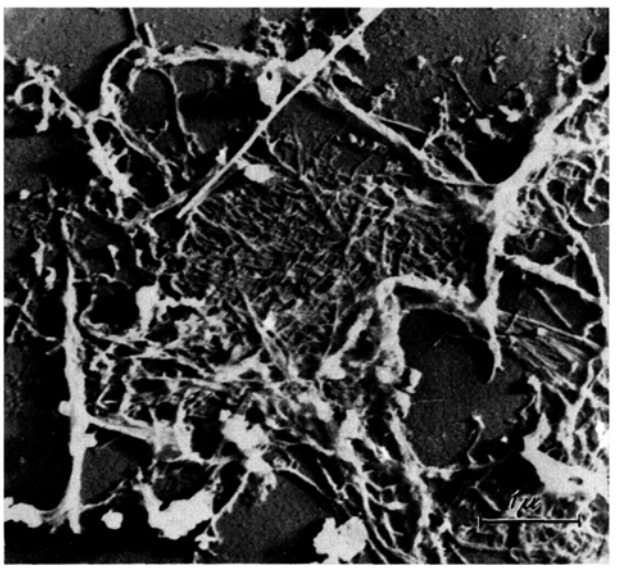


Fig. 1. – A partly disintegrated piece of primary cell wall from a viscose fiber. Magnification 13,000 ×.

Fig. 2. – Partly dissolved microfribrils. The strands have been greatly swelled by the treatment with NaOH. Magnification 12,000 ×.

the molecular elements. Such an intermediate stage is of interest in giving an indication of the way a fiber "dissolves"; through such a loosening of the molecular texture the solvent can penetrate better and the entire system must slowly disintegrate into its molecular components. These stages of breakdown are beautifully visible in the fibrils of Fig. 2. It might be expected that additional information about the internal structure of cellulose fibrils could be gained from these strongly swelled fibers. Their detailed study with this in mind gave slight and inconclusive evidence for the presence of fine-twisted threads, but was otherwise unrewarding.

The foregoing are structural elements of the original cellulose which have retained their initial form throughout the chemical treatment. Other detail seen within fibers of viscose silk can be recognized as material put into solution by the carbon disulfide. Figs. 3 and 4 show such material and emphasize the heterogeneity of the entire process of disintegration. Breakdown products no greater than, and therefore scarcely distinguishable from, the particles of the underlying collodion film are present along with many still intact pieces of fibril. The larger swelled shreds which stream in all directions from the original strands disperse gradually into discrete elements. Fig. 3 shows that this "dissolved" substance has a visible structure and must therefore consist of particles which are associations of numerous cellulose molecules.

Fig. 4 has been selected as typical of the artificial silk used in this investigation. It shows again the partially decomposed cellulose shreds interspersed with still further disintegrated fragments. Clearly these viscose fibers do not consist of completely dissolved and subsequently regenerated cellulose, but rather of such material serving as a cement and binder for undissolved components suspended in it. Fig. 5 shows a longitudinal section through a viscose fiber whose heterogeneous innerstructure is apparent only in its thin central strand. Elsewhere the surfaces are smooth because all holes and fractures have been filled in during extrusion into the precipitating bath. The big empty spaces visible in the picture probably have been produced by the action of the blendor. As TRILLAT and MATRICON[1] were able to show by diffusing air through viscose films, pores are present, but the present experiments do not throw light on their character.

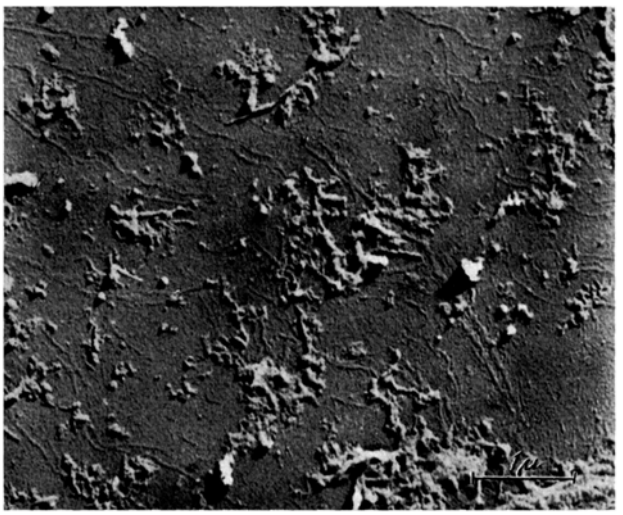


Fig. 4. – Residue of fine-grained undissolved material in a fiber of viscose silk. Magnification 13,000 ×.

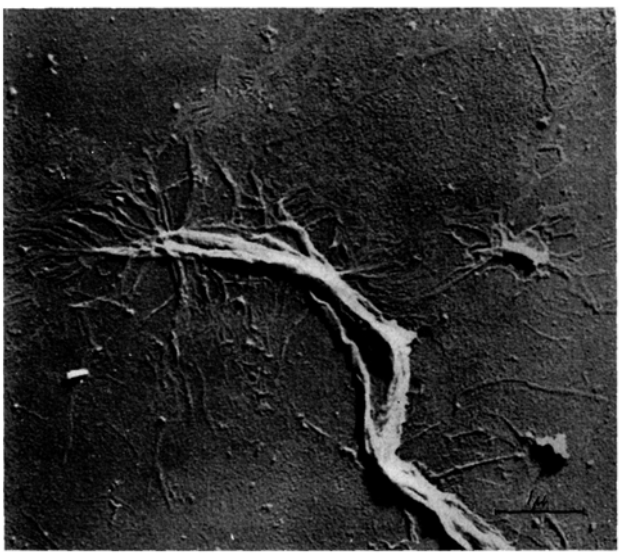


Fig. 3. – Molecular components of cellulose xanthogenate seen in a viscose fiber, together with a few strongly swelled cellulose fibrils. Magnification 12,000 ×.

Discussion

From the foregoing results it is understandable that the tensile strength of the viscose fiber should be significantly less than that of native cellulose. The mean chain length is considerably shortened during the early "ripening" process and the later "solution"; and it is scarcely conceivable that fibers built up from an admixture of regenerated, short filaments and undissolved fragments should equal in strength the original cellulose.

The electron microscopic evidence that cellulose does not go into "solution" as individual molecules but as molecular groups and larger fragments agrees with the X ray observations of HESS and TROGUS[2] and of

[1] J. J. TRILLAT and M. MATRICON, J. chim. physique *32*, 101 (1935).

[2] K. HESS and C. TROGUS, Cellulosechemie *13*, 84 (1932).

Fig. 5. – A section through a viscose fiber. Magnification 17,000 ×.

SCHRAMEK and KÜTTNER[1], who found that the technical sulfided product always shows the diffraction lines of alkali cellulose.

It would be necessary to investigate artificial silk from other manufacturers before generalizing from the results of these experiments. There is reason to believe, however, that much the same structure would be found in other viscose fibers spun in the same way, though perhaps with different relative amounts of fragments and dissolved materials.

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Zusammenfassung

Mit Hilfe des Elektronenmikroskops wurde die Feinstruktur einer Viskoseseide untersucht. Es zeigte sich, daß diese Fasern sehr heterogen zusammengesetzt sind. Wir fanden Teilchen mit typischer Zellwandstruktur und stark verquollene Fibrillen, welche in einer fein strukturierten Grundmasse, die als Auflösungsprodukt von Zellulosexanthogenat zu betrachten ist, eingebettet waren. Aus den Bildern geht hervor, daß die Zellulosemoleküle nicht einzeln, sondern gruppenweise in Lösung gehen. Beim Auspressen ins Fällbad werden diese Teilchen fixiert und bilden so den Kitt, um die übrigen Beimengungen miteinander zu verbinden. Alle Unebenheiten werden dadurch ausgefüllt, und die fertige Faser erscheint gleichmäßig glatt.

[1] W. SCHRAMEK and E. KÜTTNER, Kolloid-Beih. *62*, 331 (1935).

[2] Special Fellow of the National Institutes of Health. Permanent Address: Pflanzenphysiologisches Institut der ETH., Zürich, Switzerland.

Die Elution von Fettfarbstoffen durch Albumin und Polyvinylpyrrolidon

Im Organismus des Warmblüters finden ständig Austauschreaktionen statt zwischen Blutstrom und den angrenzenden Geweben; bald sind es Vitamine und Hormone, die durch Adsorption und Diffusion in das Gewebe übertreten, bald sind es Abbauprodukte, Pigmente und Toxine, welche das Blut durch Elution aus den Geweben herauslöst. Nachdem die Elution in erster Linie von den Proteinen des Blutserums bewirkt wird, welche anschließend im Sinne BENNHOLDS[1] den Transport zum Ausscheidungsorgan übernehmen, haben wir ein Modell[2] entwickelt, an welchem die eluierende Fähigkeit von Serumproteinen und von Plasmaersatzmitteln geprüft werden kann. Neuerdings können an der solchermaßen standardisierten Elution neben den hydrophilen Eigenschaften der Proteine auch deren lipophile Eigenschaften in meßtechnisch einfacher Weise bestimmt werden. Dazu werden aus Hautfasermembran tierischer Abkunft (nicht Cellophan, dessen Anfärbung ungenügend wasserecht ist) Scheibchen gestanzt von 9 cm Durchmesser. Entsprechende Membranen sind bei Firmen des Fleischwarengroßhandels leicht erhältlich. Es ist zweckmäßig, die Hautfasermembranen in der feuchten Atmosphäre eines Eisschrankes aufzubewahren. Die Membranscheibchen werden alsbald in Petrischalen gelegt von 9,5 cm Durchmesser und mit den folgenden Lösungen, deren Volumen stets 30 cm³ beträgt, allseitig überspült. Vorerst werden die Fettfarbstoffe zwecks Reinigung zweimal aus Wasser/Alkohol-Gemischen umgefällt; darauf stellt man Stammlösungen her von 100 mg% Sudanrot I (Grübler, Leipzig), Sudanschwarz B (Hollborn, Leipzig) und Blau BZL (Ciba, Basel) in Alkohol 96% (vgl. L. LISON[3] und B. ROMEIS[4]). Von der Stammlösung des Sudanrot I wird 1 cm³ zu 13 cm³ Alkohol 96% und 16 cm³ H_2O zugefügt; mit den übrigen Fettfarbstoffen wird analog verfahren. In diesen Farblösungen verbleiben die Fasermembranen (je eine auf 30 cm³ Farbstofflösung) für 24 Stunden (Zimmertemperatur). Darauf werden die Membranen mit der Pinzette umgedreht und weitere 72 Stunden in der Lösung belassen. Nach total 96 Stunden läßt sich aus dem Farbstoffschwund kolorimetrisch ermitteln (Korr. f. Verdunstung), daß von den Membranen 650–700 γ Sudanrot, 350–400 γ Sudanschwarz und 900–950 γ Blau BZL aufgenommen wurden. Die Membranen werden alsbald mit Wasser kräftig abgespült und sind für die Elutionsversuche bereit. Um das Ergebnis genau quantitativ zu gestalten, schreibt man von jeder Membran ihren Farbstoffgehalt auf. Für den Vergleich der Elution hydrophiler Azofarbstoffe (siehe WUNDERLY[2]) werden von Evans-Blue (British Drug House, London), Benzoblau, Trypanblau und Victoriablau (alle Grübler, Leipzig) soviel in je 30 cm³ H_2O gelöst, daß die Konzentration mol/100000 beträgt. Nach 48 Stunden sind die Farbstoffe quantitativ auf die Fasermembran gezogen; es wird kräftig mit H_2O abgespült und die noch feuchten Membranen in 30 cm³ der Elutionsflüssigkeit eingetaucht (Petrischalen, bedeckt). Diese Elutionsflüssigkeit enthält für alle Farbstoffe gleichermaßen 0,166 g% Albumin, von dem salzarmen Albuminkonzentrat, das die Cutter Laboratories (USA.) für Injektionszwecke herausbringen. Die Reinheit des Präparates wurde von uns elektrophoretisch geprüft

[1] H. BENNHOLD, H. OTT und M. WIECH, Dtsch. med. Wschr. *75*, 11 (1950).

[2] CH. WUNDERLY, Ärztl. Forschg *4*, I/29 (1950).

[3] L. LISON, Histochimie animale (Paris 1936).

[4] B. ROMEIS, Mikroskopische Technik (München 1948).