CELLULOSE CONTAMINATION: A POSSIBLE SOURCE OF ERROR IN THE INTERPRETATION OF PREVIOUS EXPERIMENTAL EVIDENCE FOR THE α-KERATIN PROTOFIBRIL

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Studies of transverse sections of osmium- and lead-stained α-keratin in the electron microscope led to the suggestion (1) that the microfibril (about 75A in diameter) contained protofibrils about 20A in diameter. Johnson and Sikorski (2) criticized this conclusion on the grounds that the high-resolution image of a microfibril in transverse section should not be regarded as a simple projection of the specimen. Subsequently, experiments were reported which appeared to show that filaments of indefinite length, and of width often as low as 20A, could be isolated from chemically modified α-keratin by ultrasonic fragmentation in formic acid (3–6). This latter evidence, therefore, provided strong support for the protofibrillar concept. However, recent studies in this laboratory indicate that the evidence for the fragmentation of α-keratin into protofibrils is not as conclusive as was first thought, mainly because no account seems to have been taken of the possibility of the presence of cellulosic contaminants in the keratin specimens.

Ohad and Danon (7) have shown that long filaments, 20–30A in diameter, can be obtained from bacterial and corn cellulose. We have also found that ultrasonic irradiation of a wide range of cellulosic materials (burr, shive, grass, cotton, wood, paper, and even dust), in water or formic acid, yields filaments apparently indistinguishable from those that have been ascribed to α-keratin protofibrils. (Compare Figs. 1–3 with: Fig. 2 *d* in reference 4, Figs. 1 and 2 in reference 6, and Plates 2 C and 2 D in reference 5.) It was also noted that many effects previously associated with filamentous material, for instance "kinking" and "repeats" (4), supposedly derived from α-keratin, have also been observed in purely cellulosic preparations. (Compare Fig. 4 with: Figs. 2 a and 2 c in reference 4, and Plate II in reference 3; and Fig. 5 with Fig. 1 a–e in reference 4.)

Cellulose contamination may be acquired during specimen preparation from cleaning tissues, filter paper, dust, etc., but, once recognized, the dangers from such sources may be minimized. A more serious problem is the appreciable amount of cellulosic plant material that wool (which has been the major source of α-keratin in the fragmentation investigations) normally contains in its raw state. Such cellulose contamination is picked up by the sheep in the form of burrs, grass, twigs, dust, etc. It is usually so firmly entangled with the wool fibers that physical removal of obvious nonwoollen substances and thorough solvent extraction, the usual methods of wool purification, are unlikely to completely remove the cellulose contamination, particularly those particles of microscopic dimensions. Indeed, when a Merino wool sample was "cleaned" by the normal methods and subsequently immersed for 2 days in several changes of a 20% solution of sodium hydroxide, a non-

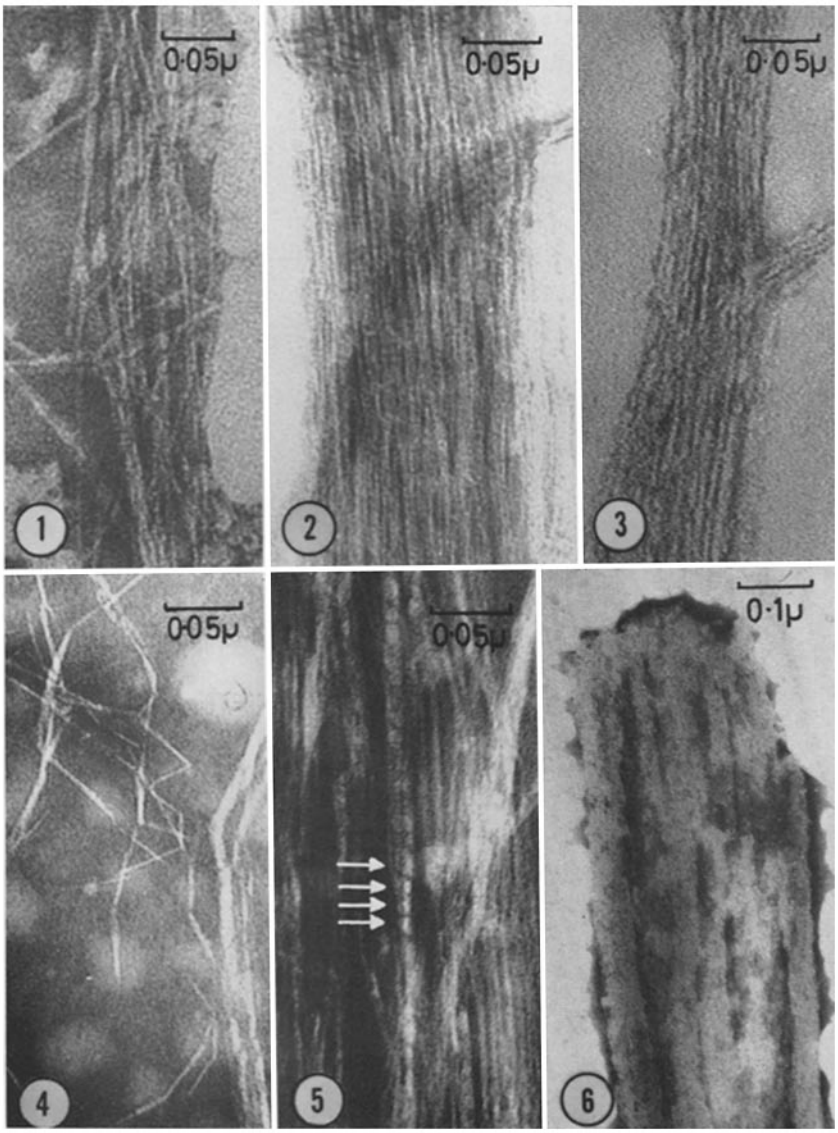

FIGURE 1 Filaments from ultrasonically disintegrated burrs, negatively stained with sodium tungstate. × 200,000.

FIGURE 2 Filaments from ultrasonically disintegrated filter paper, negatively stained with sodium tungstate. × 200,000.

FIGURE 3 Filaments from ultrasonically disintegrated "dust", negatively stained with sodium tungstate. × 200,000.

FIGURE 4 Filaments from ultrasonically disintegrated paper tissues, negatively stained with sodium tungstate. × 200,000.

FIGURE 5 Filaments from ultrasonically disintegrated shive, negatively stained with sodium tungstate. × 200,000.

FIGURE 6 Typical sheet obtained by ultrasonic fragmentation of iodinated porcupine quill, negatively stained with sodium tungstate. × 100,000.

proteinous residue remained. By weight this residue was about 0.05% of the initial sample. When the residue was disintegrated by ultrasonics, it yielded an abundance of filaments 20–30A in diameter. After staining the residue and examining it in the light microscope, we found that it consisted mainly of cellulose, thus reinforcing our doubts as to the efficiency of conventional laboratory methods of cleaning wool.

Our experiments with wool did not confirm earlier impressions of "large" yields of protofibrils (4), even after prolonged high-power ultrasonic fragmentation of treated wool (3–6). The typical appearance of an overwhelming majority of the fragments of disintegrated α-keratin is illustrated by Fig. 6. The fragments do not seem to show any significant information on the ultra-structural level, and we have not seen evidence of intermediate stages between such fragments and the filaments 20–30A in diameter. In fact, the relatively small and somewhat variable amounts of "protofibrils" found were not inconsistent with the assumption that they originated from traces of cellulose contaminant, and not from the wool.

We therefore decided to examine specimens that had been pretreated by methods designed to remove cellulose. Firstly, we used wool that had been carbonized (8), a process used industrially to purify wool by selective charring of the cellulose. It is significant that after carbonization appreciable quantities of charred material were found in what had previously appeared to be clean wool samples. The carbonized wool was chemically modified by the several methods claimed to aid the isolation of protofibrils (3–6), and then ultrasonically disintegrated in formic acid. In each case, there was a severe reduction (by an estimated factor of at least 10^2–10^3) in the already small proportion of filaments (20–30A in diameter) observed in the debris. The very rare occurrence of filaments after carbonization might well be due to incomplete removal of cellulose. The second approach to the problem was to use porcupine quill as an alternative source of α-keratin. The external layers of the quill tips were scraped away so that there was no possibility of initial contamination of the specimen by cellulose. Material purified in this way was not found to yield any evidence of protofibrils. These results therefore strongly suggest that the filamentous material previously interpreted as α-keratin was, in fact, of cellulosic origin.

In order to obtain an unequivocal answer to the question of identity of the 20–30A filaments, a method of distinguishing between small fragments of cellulose and keratin directly in the electron microscope is necessary. Attempts to use enzymes, ferritin-labeled antibodies and electron diffraction for this purpose have not proved satisfactory to date.

In summary, it would appear that fragmentation experiments purported to show isolated α-keratin protofibrils should, at the very least, be regarded with suspicion until the identity of the filaments can be proved beyond reasonable doubt. This conclusion does not necessarily mean that the protofibrillar concept should be abandoned. The conclusion demonstrates that additional evidence is required before the concept can be considered proven. Certainly, the X-ray diffraction evidence is consistent with an association of α-helices into two- or three-stranded coiled–coil ropes (9, 10) extending for some 60A in a longitudinal direction (11), and also with transverse subdivision of the microfibril into units about 20A in diameter (11). However, it is clear that there is insufficient evidence available at the present time to establish the relative arrangement of such 60A lengths of coiled coil.

Finally, it might be suggested that, in addition to the preceding remarks concerned with fully hardened α-keratin, the fine filamentous material reported to be obtained from hair roots (12), β-keratin (4, 5, 13), and silk (14) should also be interpreted with some caution until all suspicion of cellulose contamination is removed.

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