U. S. Department of Commerce
National Bureau of Standards

Research Paper RP1921
Volume 41, September 1948

Part of the Journal of Research of the National Bureau of Standards

A Method for the Electron Microscopy of Wool[1]

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A simplified method for making shadowed thermoplastic negative replicas of fibrous materials was developed. Through this technique, fine details of the morphology of the wool fiber, before and after treatment, were clearly revealed by the electron microscope. The structural implications of the electron micrographs are discussed with respect to the subject of wear and in reference to the observations of other investigators.

I. Introduction

1. The Problem

The electron microscope has been a useful tool for observing objects which are thin enough or which are sufficiently small and adequately dispersed to allow the proper scattering and transmission of electrons in the conventional 50-kv microscope.[2] The high resolving and magnifying powers of the electron microscope have thus been used with considerable success in the study of pigments, smokes, viruses, bacteria, and a variety of other colloidal particles. On the other hand, when efforts are made to study organic fibers such as wool, ordinary techniques of direct specimen preparation fail. In the first place, the fiber is too thick to transmit a sufficient intensity of electrons to excite the phosphor-coated screen into luminescence or to form an image on the photographic emulsion. At best only a profile view can be obtained when a section of the fiber is inserted in the specimen holder. Second, and no less troublesome, is the fact that in scattering most of the electron beam there is an exchange of energy. The subsequent generation of heat and electrostatic charges causes a charring and disintegration of the fiber itself. Third, the necessity of operating the electron microscope at a pressure of less than 10^{-4} mm of mercury results in dessication of the specimen. The literature of the past few years is replete with descriptions of limitations that make observation and interpretation difficult for the electron microscopist.

2. Evaluation of Existing Techniques

Partial solutions to the problem have been found by those concerned with the study of wool fibers. Fragments of triturated fibers of reduced and methylated wool are not adversely affected by the intense beam of electrons and are, in part, transparent to it. Such techniques, however, are very limited. Not only to they preclude getting detailed information about the surface structure of the fiber, but they eliminate the possibility of observing the same fiber before and after treatment because of the mechanical disorganization and chemical alteration that these techniques produce [1 to 6].[3]

The limitations of direct methods of surface observation by electron-optical means have led to the development of ingenious and diverse techniques for preparing thin film replicas of the surfaces of electron-optically "opaque" objects [7, 8]. The improvements and variations of the basic replica methods reported by a number of resourceful workers have been successfully applied to the study of many bulk materials [9, 10, 11]. None of these techniques, however, is easily applicable to the study of degraded wool fibers, since the use of excessive heat or crushing pressure in molding the replica introduces undesirable changes. Interpretation of results is often complicated by artifacts. Moreover, most replica methods involve

[1] This paper will also appear in the September issue of the Textile Research Journal.

[2] The RCA Type EMU electron microscope was used for this work.

[3] Figures in brackets indicate the literature references at the end of this paper

destruction of the specimen. This, too, eliminates the possibility of performing additional treatments on the same fiber.

In order, therefore, to augment the data on the structure of the wool fiber and to correlate these observations with results previously obtained by the direct disintegration of the fiber as well as by other methods, a simple, rapid, faithful replica technique was developed. This system of specimen preparation successfully combines methods useful for both optical and electron microscopy [12]. A negative replica of the wool fiber is made in a thermoplastic film which is thin enough to examine directly in the electron microscope and which may be shadow-cast, with a suitable metal for added contrast.

II. Replica Method

1. Materials and Apparatus

Replicas can be made with two thermoplastic materials. One, ethyl cellulose,[4] is dissolved in ethyl acetate. Addition of about 10 percent of ethyl alcohol aids solution. The other thermoplastic, which yields a slightly stronger film and one that withstands electron bombardment better, is polystyrene.[5] Styron granules "dissolve" in benzene. Warming the solvent slightly hastens the reaction. Solutions of 1 to 2 percent (by weight) seem to be satisfactory for each resin. The principal criterion is the ability of the plastic films to transmit a sufficiently intense beam in the electron microscope. The films formed by evaporation of the solution after it is poured on to standard glass microscope slides are thicker than the substrate films usually employed for electron microscopy. About three-fourths of the slide is coated with the thermoplastic resin. The excess is drained off onto a towel by placing the glass slide in an almost vertical position. After 5 minutes of drying, a slightly wedge-shaped film, averaging about 0.4 micron in thickness, is formed.

The fibers to be studied should be rinsed in ethyl alcohol and/or ether to remove surface grease and dirt.[6] After being dried at room tempreature, these fibers are then singly placed upon the thermoplastic lacquer that is on the glass slide. Another glass microscope slide is then placed on top of the fibers, sandwiching them with the plastic film. This sandwich can be firmly held together with the aid of a C-clamp and a couple of strips of cardboard and heat-resistant rubber cut to fit the glass slides. Reasonable care must be exercised not to twist the assembly out of alinement. The rubber and cardboard pads take up most of the torque and contact pressure produced when the clamp screw is tightened, by hand, to the point where the sandwich will be firmly fixed without breaking the glass and without displacing the arrangement. The clamped glass sandwich of film and fibers is then heated in a laboratory resistance-type oven for about 20 minutes at 90° C. The film is thus softened to the point where it will flow and accept the wool fiber and form a faithful replica of the surfaces in contact with it.

The variable factors that affect the quality and resolution of the plastic replica are time, temperature, pressure, thickness of the film, and diameter of the fiber. If the thermoplastic is allowed to remain in the oven for too long or at too high a temperature, too much plastic flow and deformation will result. If too much pressure is applied, the displaced material will pile up on each side of the fiber and will disturb the quality and appearance of the replica (figs. 8 and 9). If the film is too thin for the diameter of the fiber, the fiber will be pushed through it to the glass and will prevent the plastic from molding itself about the fiber surface. To achieve the proper balance of all these elements is not a difficult problem, inasmuch as the limits of control are not critical. One or two trials ordinarily provide sufficient experience so that numerous acceptable replicas can be made in a comparatively short time. After the clamped sandwich is removed from the oven and allowed to cool to room temperature, it can be separated. Usually the wool fibers come away with the uncoated glass slide, leaving semicylindrical impressions in the plastic. If not, it is a simple matter to remove them whole with the aid of a fine brush or tweezers. These fibers having once been accurately molded can be saved for further chemical or physical treatment and subsequent replication.

2. Metallic Shadow-Casting

Metallic shadow-casting adds contrast as well as a three-dimensional aspect to the surface de-

[4] Sold commercially as Faxfilm, Ethocel, Ethofoil, etc.
[5] Styron made by Dow Chemical Co.
[6] The wool used for this work had been commercially degreased. Prolonged washing was, therefore, unnecessary.

tails of the replica [13, 14]. A very thin coating of evaporated chromium is deposited obliquely upon the plastic surface. The elevations and depressions of the surface replica cast characteristic shielded radiation shadows. Where no metal is condensed, transmission of the incident energy is a maximum. Where thick elevations and ridges occur coupled with a layer of metal, electron scattering is a maximum and little or no energy is transmitted.

The glass slide bearing the plastic film containing the replicas of the fibers is placed horizontally with the film side up in a vacuum chamber where it can be shadowed.[7] To prevent any appreciable heating, the thermoplastic replicas are placed a distance of 25 cm away from a conical basket made of 30-mil tungsten wire. The basket may be formed by winding the tungsten around the pointed end of a No. 10 wood screw. Heating the wire and screw helps to prevent brittle fracture during the winding process. If instead of the 30-mil wire a twisted double strand of 16- or 20-mil tungsten wire is used, no heat is required and it is easier to follow the troughs in the screw thread. The additional surface area provided by the stranded wire is advantageous. A 6-inch length of wire is sufficient for a basket about a centimeter high with leads of adequate length. The tungsten basket is set in the vacuum chamber so that the apex of the cone points downward and is 5 cm above the plane of the replica. A charge of 100 to 150 mg of pure chromium [8] metal fragments is placed in the basket. All of the metal is evaporated slowly with a current of 25 to 35 amperes at 10 to 15 volts in about 10 minutes at a pressure of 10^{-5} mm of mercury. A 10-minute outgassing period with 10 to 20 amperes running through the tungsten wire is a desirable practice.

The uniformity of deposition of the metal as it condenses on the specimen is a function of the mean free path and the accommodation coefficient. The latter depends upon the initial temperature, the emission temperature, and the striking temperature of the molecules or atoms of the metal. Given a large accommodation coefficient and a vacuum where the mean free path is about a meter in length, the molecules will traverse the distance from filament to specimen without collision and impinge upon the comparatively cold surface of the specimen with very high energy. If these conditions are not met, the molecules of metal will not stick where they land but will migrate, find other molecules, and form agglomerations of recrystallized metal. This produces a grainy, irregular deposition. At high magnification the poorly defined shadowed areas and the graininess of the surface provide undesirable effects.

[7] The RCA EMV unit was used for this work.

[8] Electrolytically deposited Chromium about 1/16 in. thick was used.

3. Selection and Examination

The chromium-shadowed thermoplastic negative replica, still on the glass slide, is viewed in an ordinary light microscope. Photomicrographs may now be taken, in the manner of Hardy and Plitt [12]. The replica may also be projected in a photoenlarger, in the manner of Scott and Wyckoff [15]. Or, desired areas may be selected and mounted for more detailed observation in the electron microscope. In the latter case, the woven, calendered, ¼-inch stainless steel, No. 200 U. S. Standard Sieve specimen screens are centered over the chosen areas of the replica, with the aid of a low-power light microscope. The screens usually adhere to the chromium coating, especially if contact is made with the convex side. No. 100- or 150-mesh screens would offer larger areas for observation. The glass slide bearing the replica film and the specimen screens is slowly immersed at almost grazing incidence into a 9-inch crystallizing dish of fresh tap water. The thermoplastic film can be readily floated off the glass slide with little or no teasing, especially if the edges of the glass slide where the plastic may have overrun are rubbed with the side of a dissecting needle or similar tool and no water is allowed to creep up over the film. The freely floating film supporting the specimen screens is removed from the water by flipping it over and out with a piece of paper about the size of a glass slide.

The screens are thereby lodged between the film and the paper. Any short-fibered paper may be used for this purpose. The short fibers prevent wrinkling and warping upon drying. Unprinted newspaper, porous filter paper, melamine resin-bonded map paper, blotting paper, or the strips used to separate the glass in a box of microscope slides may be used with about equal success. By using paper instead of glass to retrieve the floating film, the specimen screens supporting

the selected areas of the replica can be readily removed after drying. To facilitate this, however, it is advisable to prick the film about the periphery of the screen with a needle point while the film and paper are still damp. A faster and easier technique which, however, involves some risk, is accomplished by spreading a small amount of a suitable solvent on glass and placing the dried paper in contact with it. The solvent soaks through the paper and to the plastic film. When the solvent evaporates, the paper is dried flat to the glass, the plastic is cemented to the paper, and the untouched areas of the replica supported by the specimen screens can be picked up with tweezers as easily as from a much thinner film. Obviously, if too much solvent is used the entire replica may be ruined. If a reasonable amount of caution is observed, however, this catastrophe will not occur. This technique provides a much more advantageous procedure for removing the specimen than any method of cutting or tearing the comparatively thick film from a hard glass slide or a coarse wire gauze.

Various workers in the field have suggested that a possible improvement in the technique for selecting and mounting the replica film on a specimen screen could be made by placing the film, replica side up, over a piece of 200-mesh wire cloth. After scanning the film for suitable areas with a light microscope, an auxiliary mount in the revolving nosepiece fitted with a cutting die could be turned into place and the tube racked down. In this manner, the desired area of film may be punched out with a supporting screen attached. The adaptation of a microscope-punch combination seems worthwhile not only for this work but in many other aspects of electron microscopy where systematic scanning is required for selecting areas of a prepared specimen.

III. Application and Interpretation

The shadowed replica method was applied to the study of wool fibers as reflected by the characteristic changes after varying amounts of abrasion produced by an apparatus designed by Schiefer [16]. During the abrasion cycle fiber fragments fall from the abradant. This "debris", as well as the fibers bordering the hole worn through the woolen fabric, were examined. Hundreds of replicas were made of fibers abraded in this way. For comparison, replicas were made of untreated fibers, khaki-dyed fibers, as well as fibers subjected to chemical attack. The shadowed replicas were examined in the electron microscope at low magnifications obtained without the projector polepiece, as well as at the higher ranges of magnification. Electron micrographs were made with a 1-mil objective aperture and with 5- to 10-second exposure. Contact photographic reversals were made on another medium lantern slide plate for further optical enlargement. Negative prints are shown in the figures. For proper interpretation of the surface features of the negative replicas, they may be viewed as though they were obliquely illuminated. The "diameter" indicated in the micrographs represents 80 to 90 percent of the actual diameter of the wool fiber.

A wool fiber when plucked from the living animal consists of two distinct regions. The root is the living part and grows from the hair follicle. The nonliving portion that emerges from the root is called the shaft, which is the section shorn from the sheep and is here designated the wool fiber [17]. It generally consists of three principal concentric layers—a thin outer covering of scales (cuticle), a middle region (cortex), and a central core or pith (medulla). The relative thickness of each layer varies from fiber to fiber, as well as from sheep to sheep. In the very fine wool fibers, the medulla is often absent, and a few comparatively large scales encircle the cortex. The spindle-shaped cortical cells comprise the main bulk of the fiber. It is well known that the ability of wool fibers to felt, tangle, and curl is dependent upon the direction and shape of the imbricated scale structure and that this cuticle serves to protect the fiber from chemical and mechanical degradation (figs. 1 and 8). It has been recently suggested by Mercer et al. [2, 3, 4], that these three dominant cellular structures are encased in an amorphous material that functions as a matrix and as an intercellular cement and that the cuticle layer may be covered by this same featureless keratinous material (figs. 1 to 6). These investigators and others have shown this amorphous plastic outer covering of the scales to be readily digested in an enzyme as is the featureless intercellular cement that exists between the scale and the cortex, as well as between and within the cortical cells themselves. The similar reaction to enzymic treatment suggests similar composition. Microscopy alone cannot disclose whether there

exists a continuous matrix of a single substance that envelopes the differentiated cell structures or whether the keratinous material is made up of different protein molecules with varying properties.

The present study confirms, at least partially, the "matrix theory" of the morphology of the wool fiber. Electron micrographs of the surface replicas of mechanically abraded and unabraded wool fibers (shown in figs. 1, 2, and 3) reveal that the scale is heterogeneous in structure and consists, at least, of a double layer. The outer layer is a smooth covering for the under structure and shows no specific surface features (fig. 1). When this lacquer-like coating is removed by mechanical abrasion, a rigid striated structure is exposed (fig. 2). These regular corrugations (ridges and furrows) run more or less parallel to the longitudinal axis of the fiber. They are a feature of the scale itself and are not dependent upon, or a reflection of, the size and formation of the cortical cells. This is borne out by the fact that the ribbed structure is seen in the overhanging portions of the scales under which there is no cortex (fig. 3). With greater degrees of abrasion, the scales are polished away leaving a smooth filamentary structure with slight vestiges of the former imbricated surface (fig. 4). Still more rubbing removes the next amorphous layer under the scales and reveals the cortical cells themselves imbedded in the cementum (fig. 5). After such severe abrasion, the wool no longer manifests its characteristic filamentary structure and, as such, is destroyed (fig. 6).

Thus far the present investigation indicates that the weaving process introduces very little change in the surface structure of the fibers. Some portions show degradation of the scale, but this is by no means typical. When compared with unwoven, undyed fibers, less than 10 percent of the fibers studied showed any modification (fig. 7). These alterations are very likely associated with the previous history of the fiber. It is not necessarily a result of the weaving process. In the case of khaki-dyed woolens, similar evidence was noted (fig. 9). In a few samples where the fibers were not washed in alcohol, some crystalline material was found inbedded in the replica. This may have been some of the dyestuff that had lodged on the outer scale surface. In the case of fibers that had been chlorinated in a 1-percent aqueous solution of calcium hypochlorite long enough to wet its entire length and rinsed in a large quantity of cold water (figs. 10, 11, and 12), the sacs and blisters formed (probably the Allwörden reaction) indicated a violent attack on the amorphous lacquer-like layer of the scales. The general erosion that took place exposed the characteristic striated substructure of the scale that withstood the action of chlorine. The exposed scale edges showed the greatest amount of attack (fig. 12). The treatment given the fibers was, of course, much more drastic than that used in some commercial processes for shrink-proofing woolens.

With the foregoing method, the structural changes in textile fabrics resulting from various physical and chemical factors can be studied. Alterations in the physical structure brought about by such means as abrasion, flexion, tension, acid, alkali, chlorine, heat, sunlight, and other elements of degradation make wear a complex phenomenon with a large variety of contributing factors. Before statistical correlation of any microscopic observations of laboratory wear versus actual wear may be obtained, a large number of tests interpreted against a broad background of performance in service is necessary. The "polishing" produced with Schiefer's uniform abrasion machine offers a systematic approach to a microscopic study of a worn fabric. The characteristics of fiber structure may in turn be correlated with the morphological modifications resulting from actual wear in service. Although the fundamental problem of the molecular structure of organic fibers cannot be solved by electron microscopy alone, considerable information may be determined that is of interest to the textile technologist and to those concerned with the subject of wear resistance.

The authors acknowledge the very valuable assistance of Esther Golovato, in processing the many photographs, and of H. F. McMurdie and W. D. Appel, without whose able direction much of this work could not have been done.

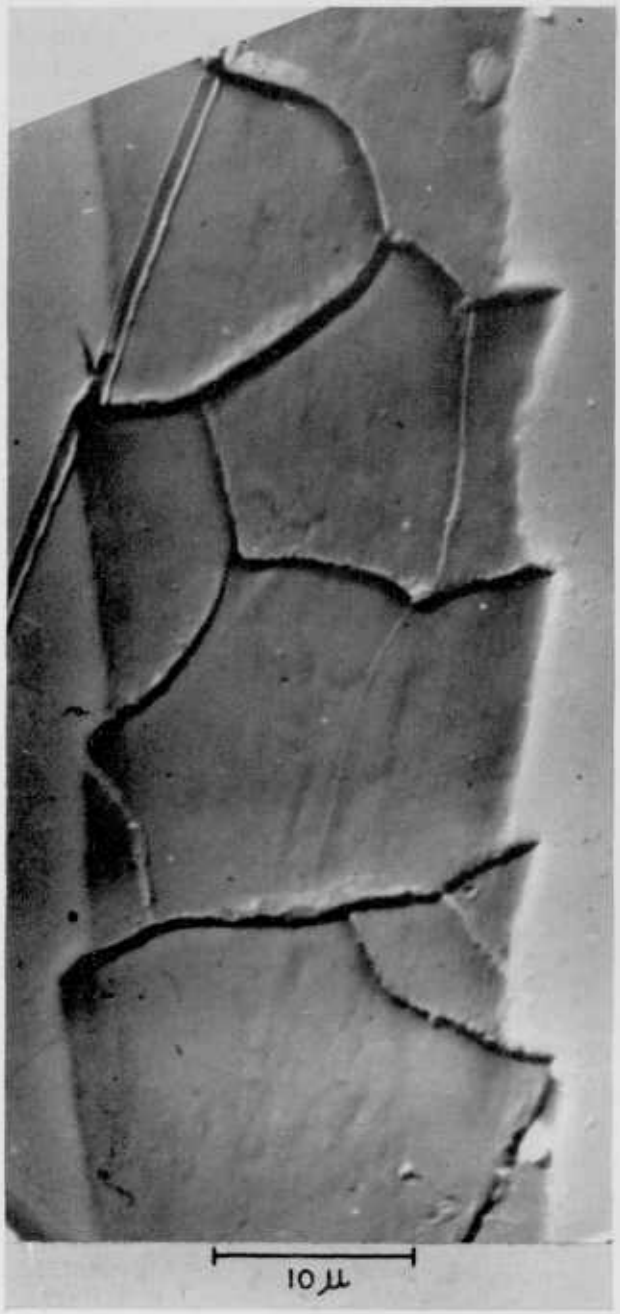

FIGURE 1.

Total magnification approx. × 2200

(electronic, × 716; optical, × 3.8).

Typical chromium-shadowed polystyrene negative replica of a wool fiber taken from unabraded, undyed, fine worsted fabric. The normal appearance shows the smooth lacquer-like coating on the imbricated cuticle. Faint striations may be seen that are part of the corrugated scale structure lying below this amorphous outerlayer.

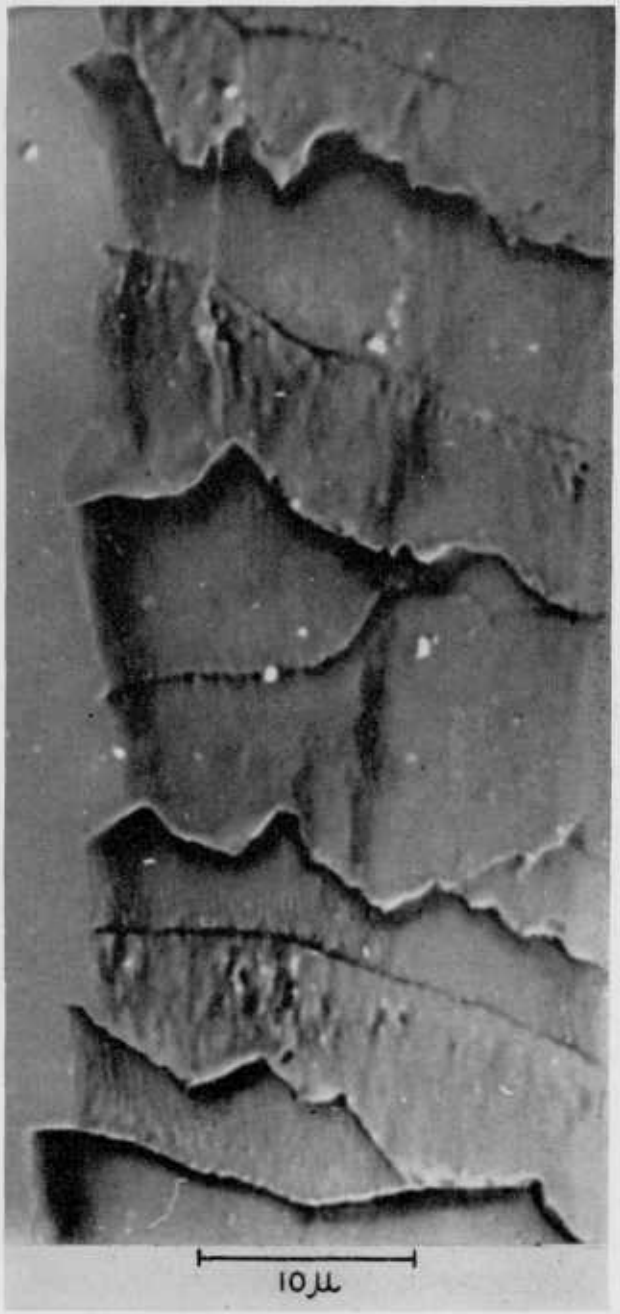

FIGURE 2.

Total magnification approx. × 2600

(electronic, × 716; optical, × 4).

Chromium-shadowed polystyrene negative replica of a wool fiber taken from a mechanically abraded fine worsted fabric. The slight amount of abrasion removed the lacquer-like outerlayer of alternate areas. The middle area was protected from wear by the interlaced nature of the fabric.

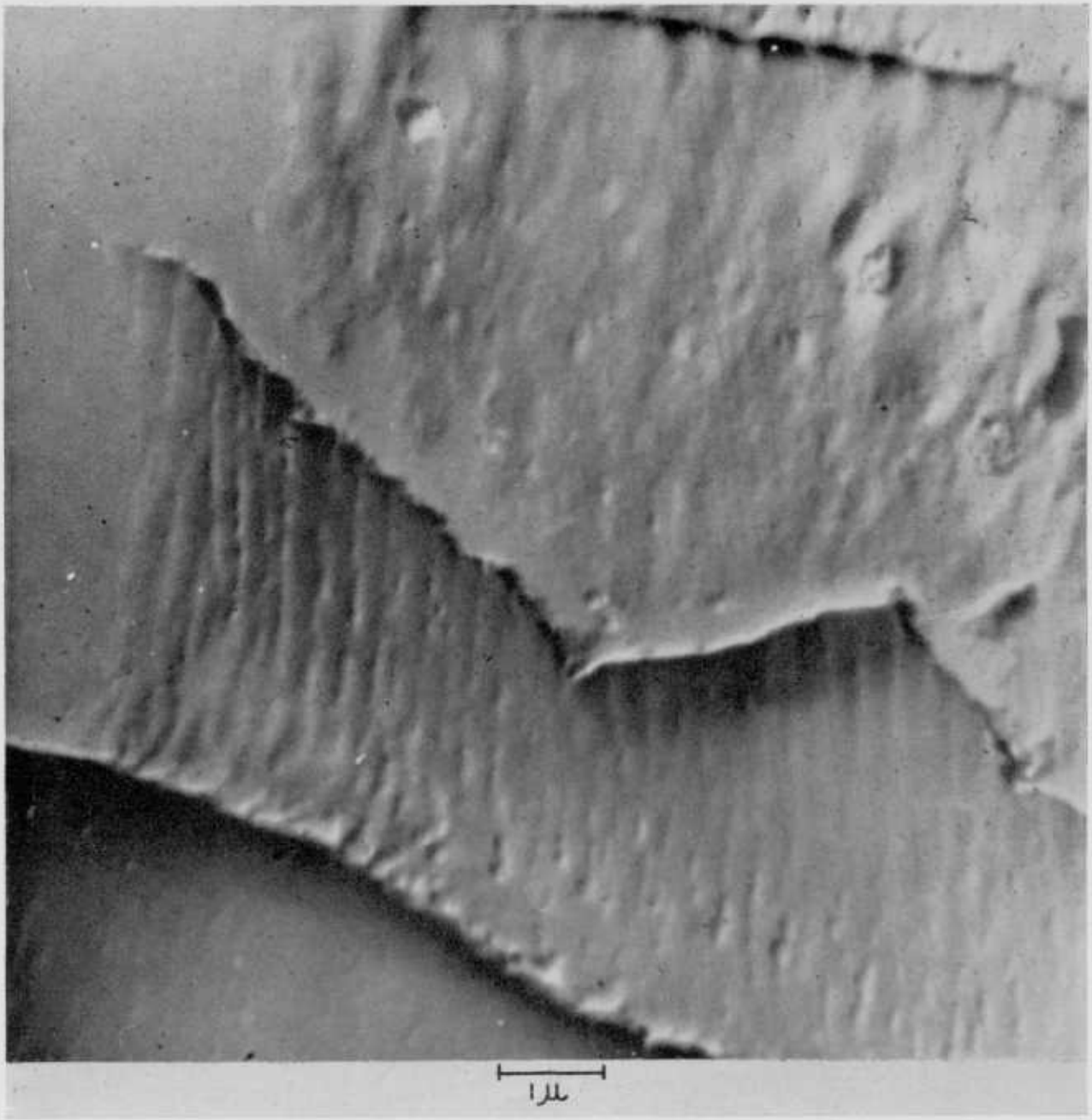

FIGURE 3.

Total magnification approx. × 16,500

(electronic, × 3600; optical, × 4.6).

An enlargement of the abraded area in the lower part of figure 2 showing the corrugated or ribbed underlying structure of the cuticle, in contrast with the smooth surface of the unabraded fiber (lower left). The longitudinal striations are an integral part of the scales themselves and not a reflection of the cortical cell formation underneath. Note the overhanging portion of a scale, in the upper left area of the replica, under which there is no cortex.

FIGURE 4.

Total magnification approx. × 2800

(electronic, × 716; optical, × 4).

Chromium-shadowed polystyrene negative replica of a wool fiber taken from the "debris" of mechanically abraded fine worsted fabric. Except for a few remnants, the scales have been removed and another comparatively smooth layer of material, the intercellular cement, lying between the cuticle and the cortex is exposed.

FIGURE 5.

Total magnification approx. × 2400

(electronic, × 716; optical, × 3.4).

Chromium-shadowed polystyrene negative replica of a wool fiber taken from the "debris" of mechanically abraded fine worsted fabric. Some scale remnants may be seen, but for the most part the coarse striated structure is that of the cortical cells beneath, indicating a greater degree of abrasion than that shown in figure 4.

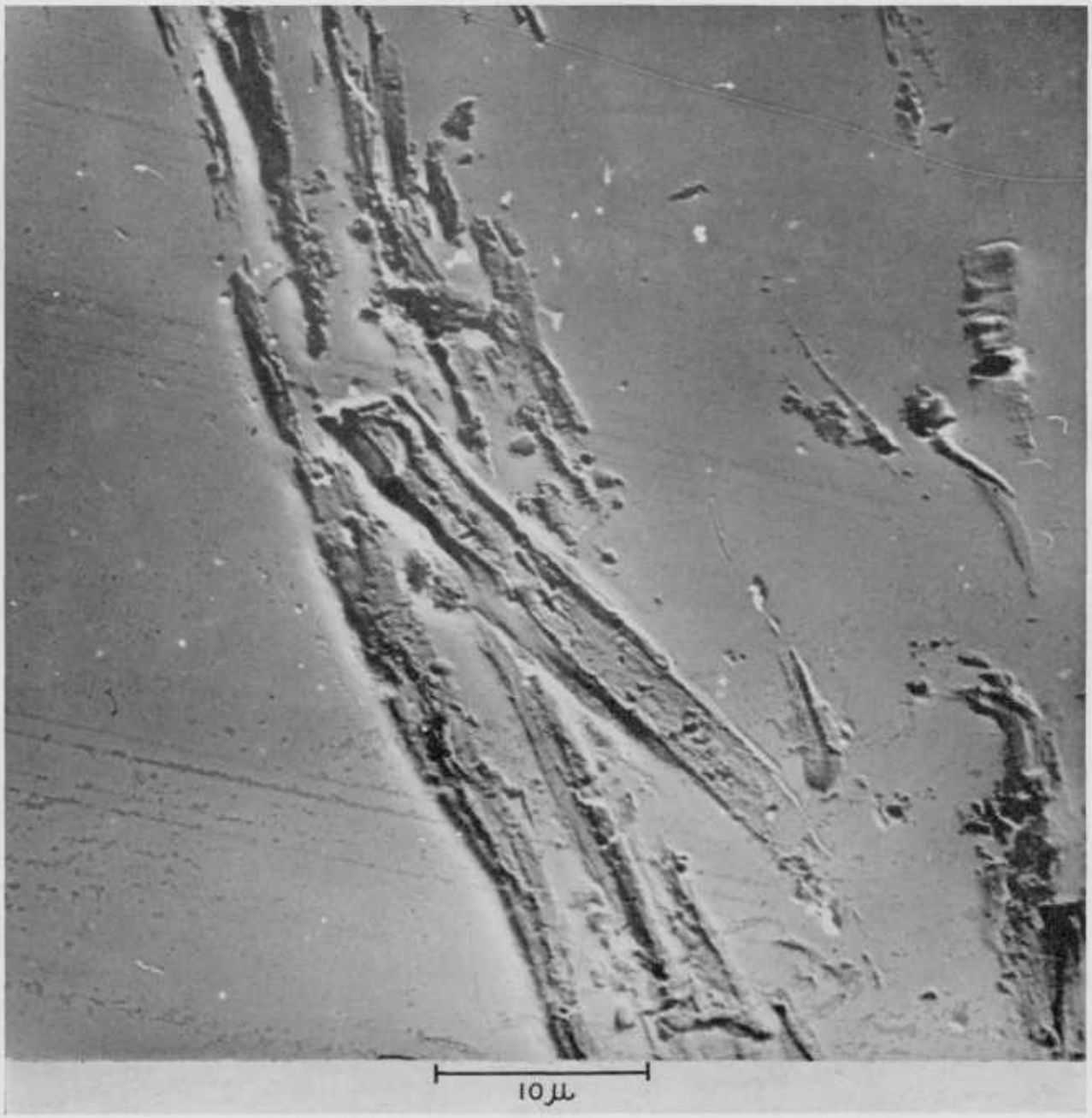

FIGURE 6.

Total magnification approx. × 3300

(electronic, × 716; optical, × 4.6).

Chromium-shadowed polystyrene negative replica of the remains of a wool fiber from severely mechanically abraded worsted fabric. The size of the structure lying across the center is about that of a cortical cell. Inasmuch as the soft cortex is easily deformed and the fiber is in the last stages of its filamentary formation, interpretation is questionable.

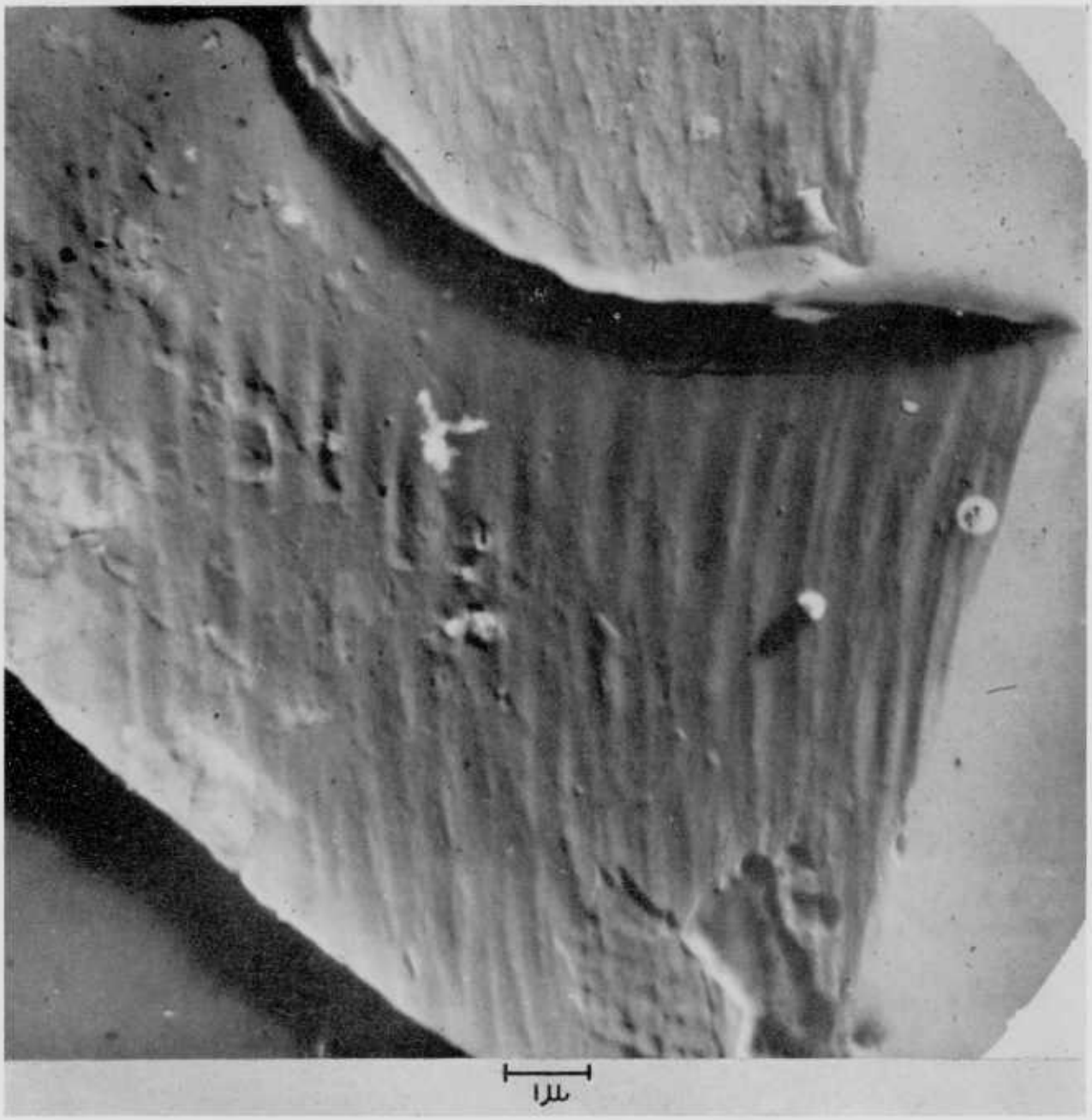

FIGURE 7.

Total magnification approx. × 13,300

(electronic, × 3600; optical, × 3.7).

Chromium-shadowed ethyl cellulose negative replica of an atypical fiber taken from unwashed, undyed, worsted yarn showing slight surface degradation associated with the previous history of the wool. Note the undegraded, smooth scale surface in the lower left corner. Here the thicker lacquer covered scale is more deeply imbedded in the thermoplastic film

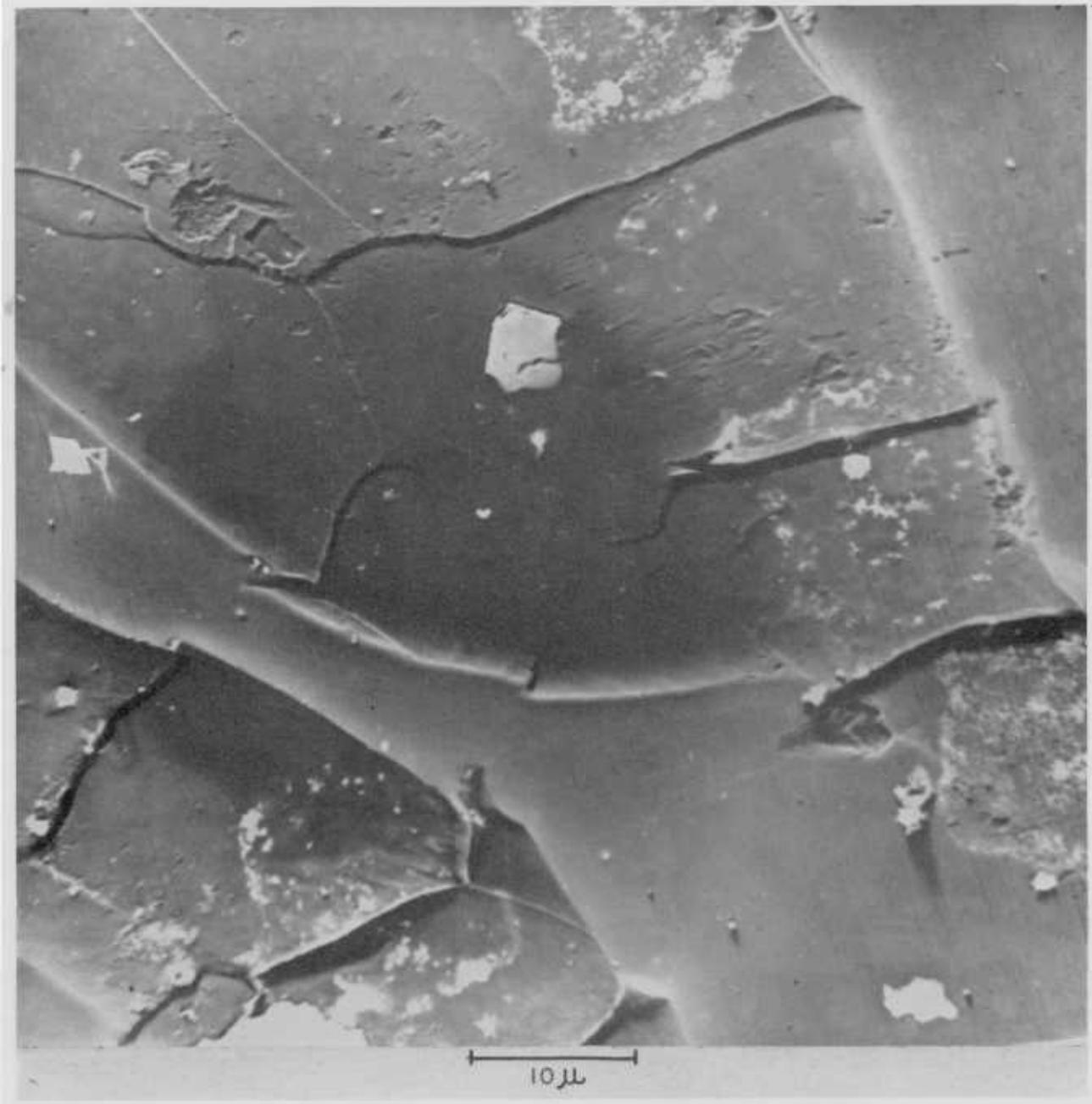

FIGURE 8.

Total magnification approx. × 2600

(electronic, × 540; optical, × 4.8).

Chromium-shadowed ethyl cellulose negative replica of two fibers from an unwashed, undyed, worsted yarn showing the bulging distortion of one resulting from excessive contact pressure on the fiber. Note that the scale structure has stretched to more than double its surface area in order to accommodate the deformation without rupture. The smooth outerlayer is still intact, indicating its plastic nature.

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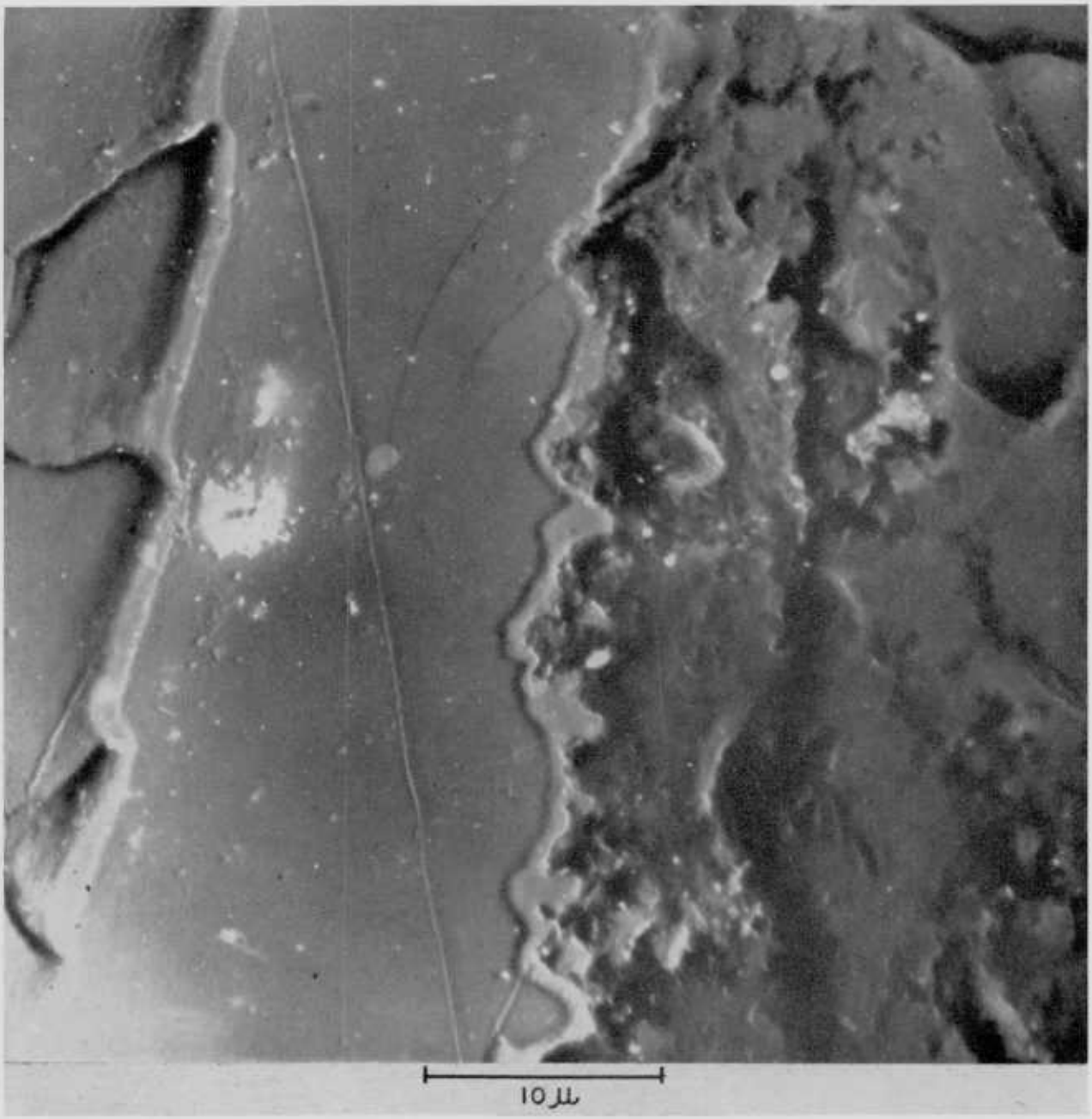

FIGURE 9.

Total magnification approx. × 3600

(electronic, × 716; optical, × 5).

Chromium-shadowed polystyrene negative replica of wool fibers taken from untreated khaki-dyed fabric. The normal surface structure of one fiber may be compared with the features of one damaged by an unknown agent, probably chemical.

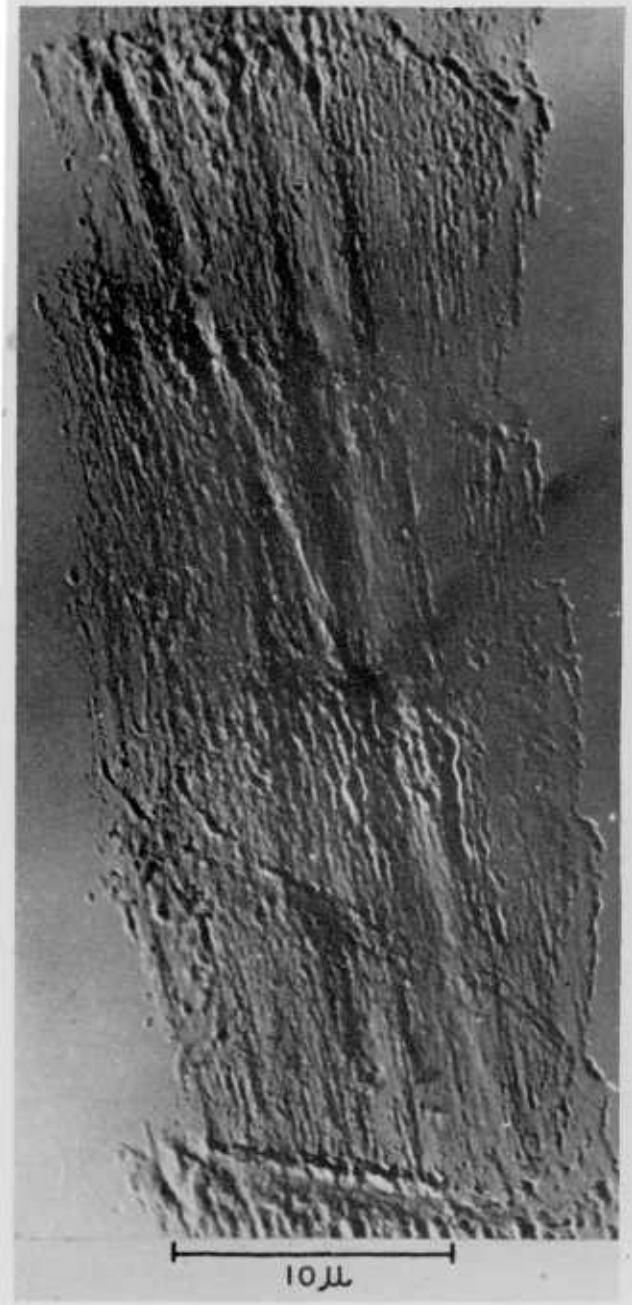

FIGURE 10.

Total magnification approx. × 3600

(electronic, × 716; optical, × 5).

Chromium-shadowed polystyrene negative replica of a fiber taken from undyed worsted fabric, immersed in a 1-percent aqueous solution of calcium hypochlorite long enough to wet it, and then rinsed in cold water. The fiber still exhibits its filamentous structure.

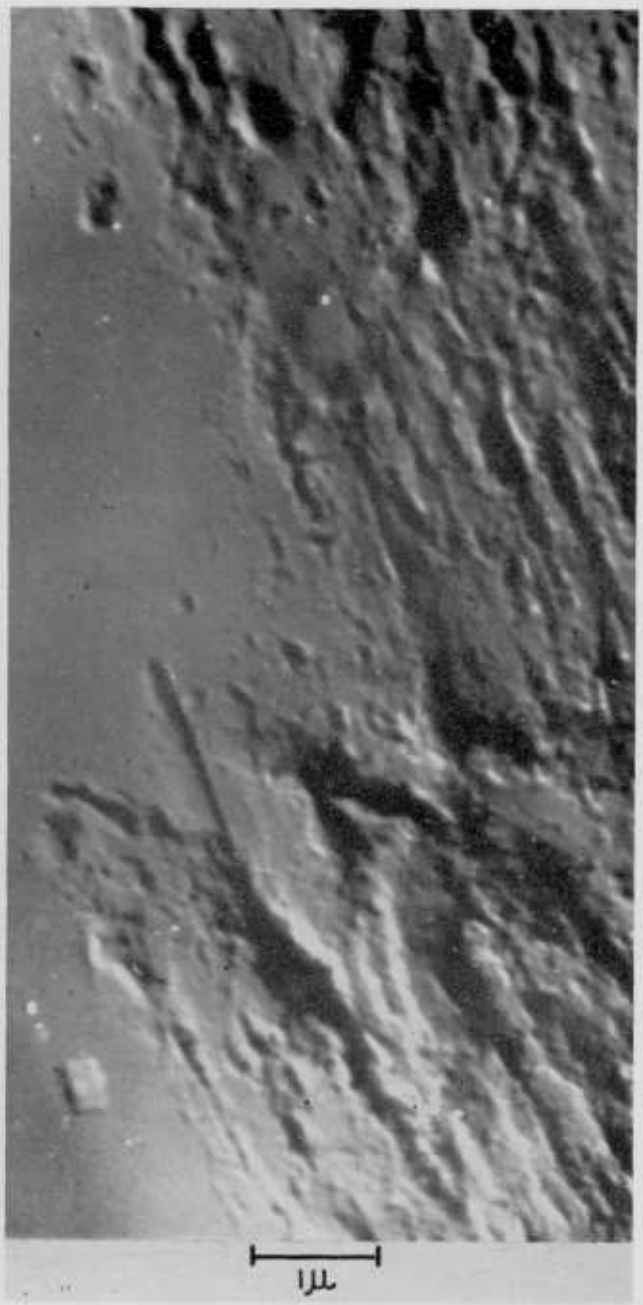

FIGURE 11.

Total magnification approx. × 17,000

(electronic, × 3600; optical, × 4.7).

An enlargement of the area in the lower part of figure 10. The erosion of the superficial lacquer-like coating exposed the characteristic striated substructure of the scales that was more resistant to the chemical attack.

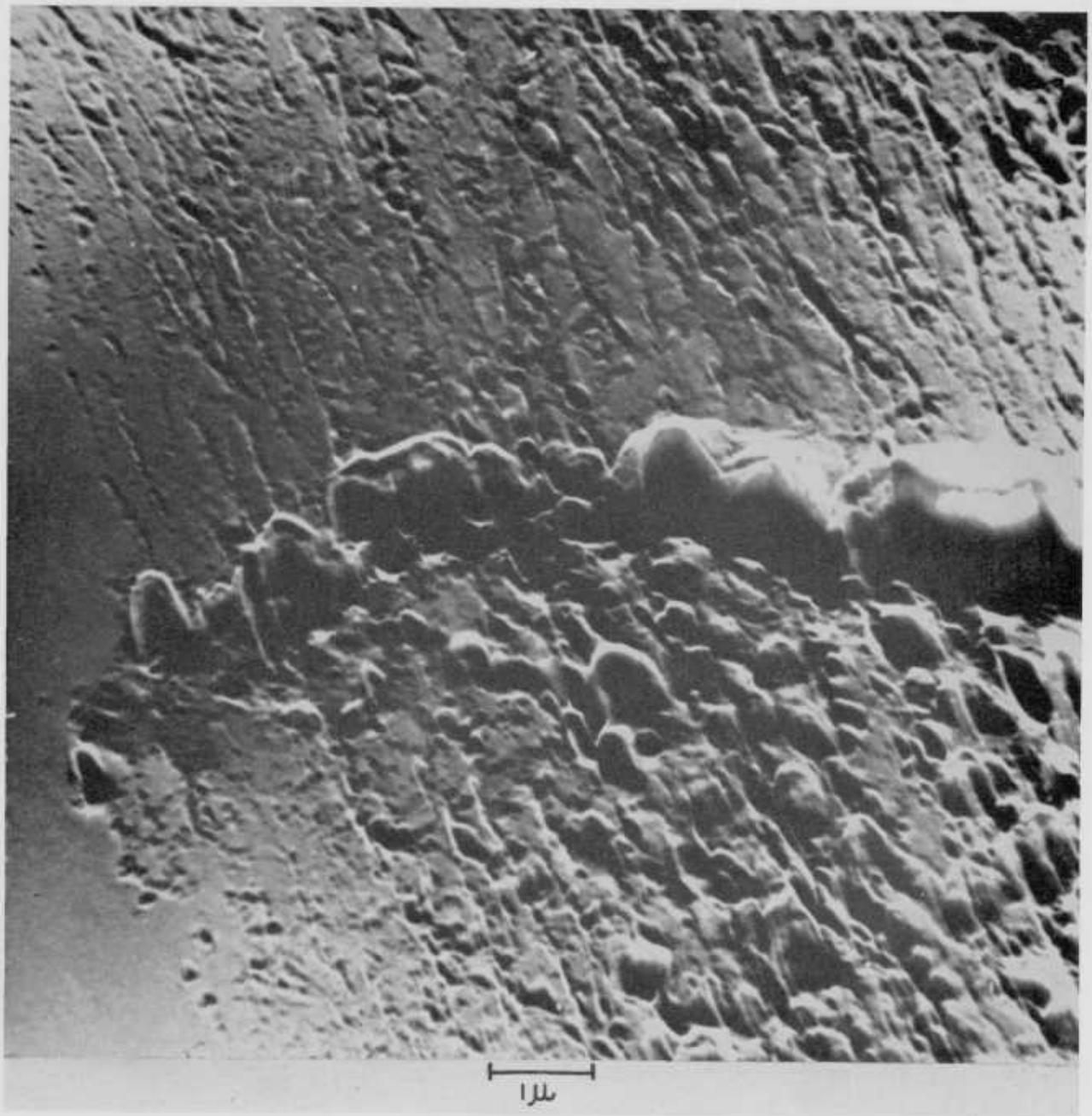

FIGURE 12.

Total magnification approx. × 16,500

(electronic, × 3600; optical, × 4.6).

Chromium-shadowed polystyrene negative replica of a wool fiber after chlorination, showing what is probably the blistery sacs of the Allwörden reaction on the lacquer-like scale surface with evidence of concentrated attack at the edges of the scale.

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WASHINGTON, June 1, 1948.