THE ELECTRON MICROSCOPE—A TOOL FOR THE STUDY OF HAIR FIBERS*

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THE ELECTRON microscope really needs no introduction because it has been in use for a number of years. During these years it has been applied to a wide variety of problems, but the application of electron microscopy to hair and related problems has received little attention (1, 2, 3). It is the main purpose of this paper to point out to those interested in hair and hair products the potential usefulness of certain techniques of electron microscopy.

The electron microscope makes available certain magnifications that could not previously be obtained by other methods. In terms of resolving power, it bridges the rather large gap between the magnifications available with light microscopy and those available with x-rays. Figure 1 shows this in a better way. This drawing has been constructed to show the structural details of a single hair fiber by the device of increasing the magnification from left to right. For those who are not familiar with the hair structure it consists of a thin layer of cells on the outside called the cuticle, and a central cortex composed of spindle-shaped cortical cells. These are 7–10μ wide and 100μ long. Within these cortical cells one finds macrofibrils. At still higher levels of magnification one notes that these macrofibrils are composed of still smaller fibrils called microfibrils. The light microscope can be used only to a magnification of about a thousand times, so that the cuticle, cortex and sometimes cortical cells can be distinguished, along with scale patterns on the surface. X-rays, on the other hand, can tell us something about atomic and molecular arrangements at a magnification of about a million (10^6). X-rays do not give a picture as the electron microscope does, but information can be derived from the patterns.

The development of the electron microscope has made possible the observation of the histological structures intermediate between cortical cells and molecules by making available magnifications between 1000 and 100,-

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1000. Even in its lower range of magnification, where electron microscopy overlaps light, the electron microscope brings out details which cannot be clearly observed with light microscopy. That is, the usefulness of the electron microscope for hair is not confined to the examination of structures in the range shown in Fig. 1, but can be used to greatly magnify those features of the hair which are ordinarily visible in a light microscope.

Since it is now possible to examine the fine structure of hair, I would like to discuss several techniques which can be used, the kinds of problems to which these techniques can be applied, and show some examples of the kind of electron micrographs obtained.

Of the various techniques which have been developed for use in electron microscopy, we believe three of these basic techniques of sample preparation to be the most useful for the study of hair. These are the replication technique for examining surfaces, the ultrathin sectioning technique for examining interior histological structures *in situ*, and the degradation technique for looking at specific histological structures as individual particles separated from the other structures.

Replication

Of the three, the replication technique is probably the simplest to use. A thin, electron-transparent cast is made of the hair surface and examined. There are many replication techniques in existence today which have been developed to examine specific kinds of surfaces. Of these techniques, we believe the one using evaporated metal to be generally the most satis-

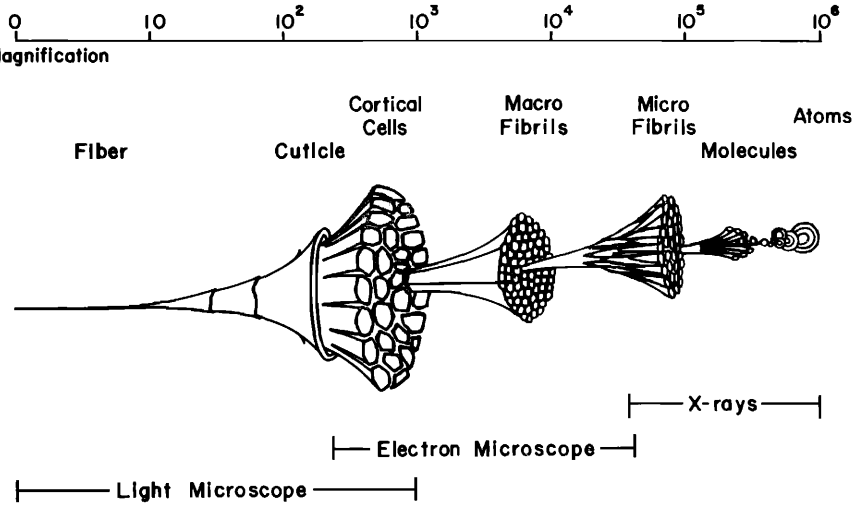

Figure 1.—Diagram of a single hair fiber progressively magnified to show the histological structure. Magnifications are only approximate. The useful range of the light and electron microscopes and of x-rays is shown.

factory. The metal replica is prepared by vacuum evaporating chromium or a platinum alloy onto the hair surface. The metallized surface is pressed into a nitrocellulose film and the hair stripped away, leaving the metal supported on the plastic. A section of the plastic is mounted on a specimen support screen and the plastic carefully dissolved, leaving the metal replica to be examined.

The plastic replica is prepared by clamping hairs against a thin film of polystyrene on glass. At slightly elevated temperature the polymer softens and takes the contours of the sample; so that, when the hair is stripped off, a negative impression of the surface is left. This must then be "shadowed" with metal to provide adequate contrast. This technique provides less satisfactory replicas than metal for revealing fine detail, but has its place when it is suspected that the hair sample may be altered by being subjected to high vacuum.

Replication has several applications in the study of hair. It is eminently suited to the examination of the surface, for example, to look at the arrangement and conditions of the scales. Once this has been determined for normal hair, the effect of various chemical or physical treatments upon the scales can be determined. Some of these effects are visible with the light microscope, but the resolution is considerably better with electron microscopy. If one then knows the surface appearance of the hair on a microscopic level, it may be possible to relate this surface condition to some of the bulk properties of the hair, such as luster, static charge production or other such properties.

There is one other special use of this technique although it can be some-

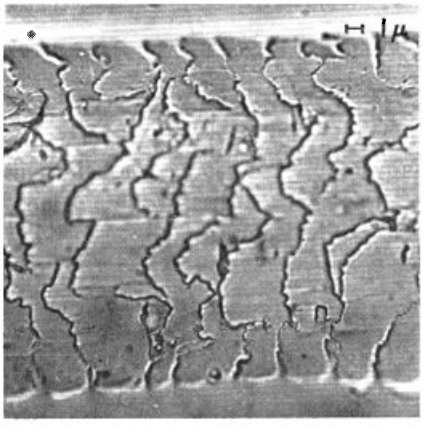

Figure 2.—Light micrograph of a plastic replica of a hair surface. This was taken at the highest magnification obtainable with light microscopy.

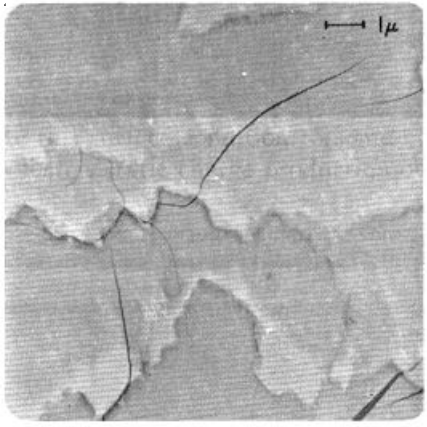

Figure 3.—Electron micrograph of a plastic replica of a hair surface.

what more time-consuming. This is replication of the cut ends of hair to give a cross sectional view. This is done in the same way as the normal method except that cut and treated surfaces are replicated. This method can be useful in the examination of the effect of reagents upon the internal histological structures of the hair, for example, determining which structures are attacked by a given reagent.

In the next few figures are shown some examples of the kind of things one observes using these techniques. Figure 2 shows a light micrograph of a plastic replica of a hair surface for comparison with the electron micrographs. The magnification here is about the limit of light microscopy, that is, about 1100×. The characteristic patterns of the scale edges can be observed, but very little is visible on the surface of the scales. Figure 3 is an electron micrograph of a plastic replica of a hair surface. This is at a fairly low magnification of 4200× but note the increased detail of the scale edges. The surface of the scales still shows no apparent structure. The cracks are artifacts in the replica. Figure 4 is an electron micrograph of a metal replica of a hair surface. Note the greatly increased definition over the plastic replica in Fig. 3 and especially over the light micrograph. The scale edges are very prominent here. The scale surfaces are relatively smooth with some evidence of particulate matter. The large particles along the scale edges may well be particulate soil or hardened oil deposits. Figure 5 is an illustration of what can be observed with regard to the effect of chemical agents on the surface of the hair. This particular sample was treated with ammonium thioglycolate under extreme conditions. Magnification here is about 4200× and furrows can be seen developing on the

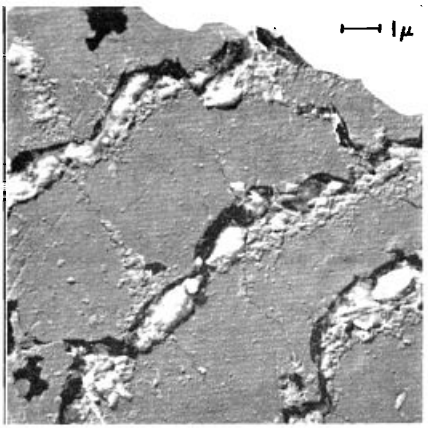

Figure 4.—Electron micrograph of a chromium metal replica of an untreated hair surface. Note increased definition of the scales over that seen in Figures 2 and 3.

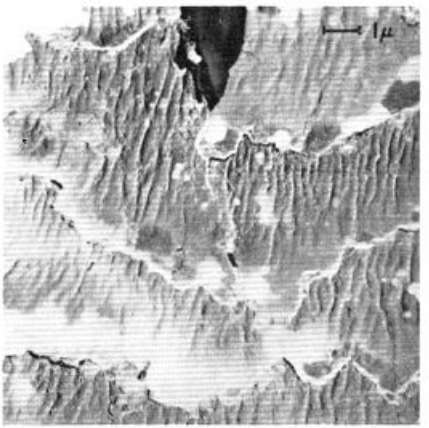

Figure 5.—Electron micrograph of a chromium metal replica of the surface of a hair fiber treated with ammonium thioglycolate under extreme conditions.

previously smooth scale surfaces. This suggests that proteinaceous material has been dissolved out of the surface layers.

Figure 6 is an illustration of what can be observed when replicas are made of cut ends of hair. This particular replica was made after etching the end of the hair with hydrochloric acid. The magnification here is considerably more than in the previous figures, about 8000×. The cell membrane of cortical cells is plainly visible, and one cortical cell is outlined in its entirety. In the center is what appears to be the dead nucleus of this cortical cell. Also faint outlines of macrofibrils are visible just below the nucleus.

These examples are just a few of the many effects which can be observed in hair by this replication technique.

Ultrathin Sectioning

The second technique useful for hair studies is that of looking directly at ultrathin sections of hair. In light microscopy thin sections are of the order of a few microns thick. In electron microscopy these sections must be a few hundredths of a micron thick because of the lesser penetrating power of electrons compared to light. There are microtomes for cutting ultrathin sections which have become commercially available in the past couple of years. Our microtome happens to be a Sjostrand thermal advance device, but a Porter-Blum mechanical advance microtome is also

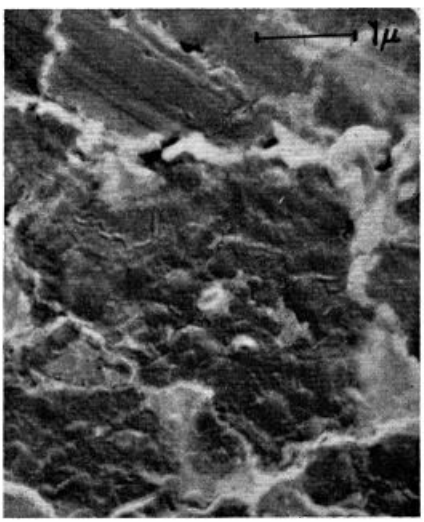

Figure 6.—Electron micrograph of chromium metal replica of an end of a hair fiber etched with dilute hydrochloric acid. Cortical cell walls, a nucleus and macrofibrils are visible.

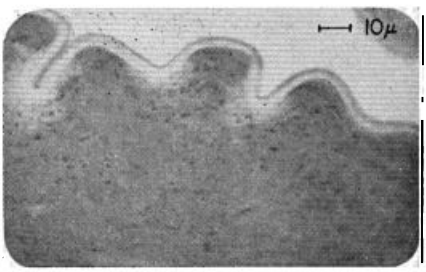

Figure 7.—Light micrograph of a cross section of hair. Pigment granules and cortical walls are visible but no structure can be discerned in the cuticle.

suitable. For cutting thin sections of hair, the hair is embedded in a polymer of suitable hardness and is cut in the microtome using either a glass or a specially sharpened metal knife. The cut sections of the single hair fiber can then be examined directly in the electron microscope.

This technique is useful for the examination of the fine histology of the hair and has the advantage that the hair need not be disintegrated to examine the histological structures as has been done in the past. The effect of all kinds of reagents upon internal structures can be determined. This technique has an advantage over the replication method in that the hair itself is being examined and not a cast of the hair. For histological studies in the hair follicle, the sectioning method can be used to great advantage to study the keratinization process. Just recently the formation of pigment granules in melanocytes near the follicle has been studied by this method and reported by Mercer, *et al.* (4).

There are two examples of ultrathin sections as illustration. First, however, Fig. 7 shows a light micrograph of a thin section at high magnification so that we can compare the electron micrographs to it. Very few fine histological details can be observed on this section. The magnification is about 760×, slightly less than the maximum obtainable. The cuticle of the hair can be distinguished along the edge but no structure is apparent within it. Pigment granules are also visible as small dark dots. Cortical cell walls are visible as lighter lines in the red background caused by staining. Figure 8 is an electron micrograph of an ultrathin section of hair at about 4300× magnification. It is seen that the cuticle is composed of thin cells that are arranged concentrically around the cortex of the hair. The cortical cell walls are very prominent. The dark spots are pigment granules. Sev-

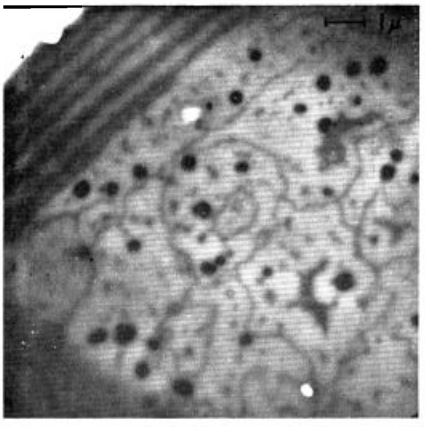

Figure 8.—Electron micrograph of ultrathin cross section of hair. Cortical cell walls, pigment granules, cuticle cell walls, and cortical cell nuclei are visible.

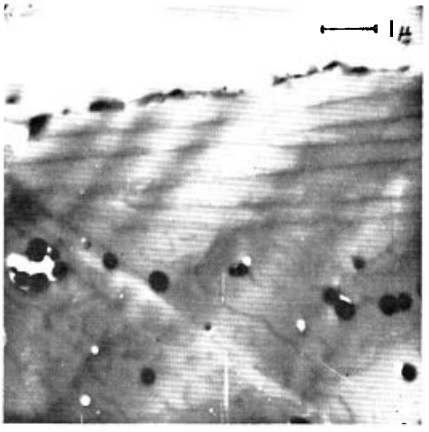

Figure 9.—Electron micrograph of ultrathin cross section of hair. Note the overlapping of cuticle cells.

eral nuclear remnants are also visible within the cortical cells. The second ultrathin section (Fig. 9) is interesting because it shows the way cuticle cells overlap.

The ultrathin sectioning technique has one disadvantage in that the various hair structures may not be adequately resolved. This is because the chemical composition of these structures is so similar that electrons may penetrate them to about the same extent, giving little contrast in the micrograph. Potentially, however, the ultrathin sectioning method shows much promise for studying hair by electron microscopic techniques. It has the advantage, as mentioned previously, of looking directly at the interior of the hair as it occurs in nature.

Degradation

The third method useful for hair studies is the method of degradation to get separate particles of actual hair material. This method involves breaking the hair down into its various histological components by a digestion, then examining the debris from the digestion in the electron microscope.

Several reagents may be used for the digestion, for example, enzymes, acids and strong bases. Enzymes like trypsin are more suitable than strong acids or bases because they act more slowly and separate the hair into more distinct structures. This allows one to stop the degradation at any desired level of complexity and examine the resulting separate histological structures.

This method is useful for examining the separate components of normal hair and the effect of reagents upon these structures. It has a serious dis-

Figure 10.—Electron micrograph of fibrillar material from a trypsin digestion of hair. Macrofibril is breaking down into microfibrils.

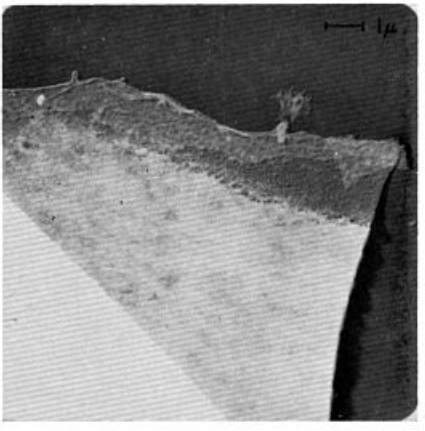

Figure 11.—Electron micrograph of a scale (cuticle cell) fragment from a trypsin digestion of hair.

advantage in that one is never sure that any changes observed are due to treatment with reagent or due to the degradation process itself. A further difficulty is that the degradation products, in order to be shadow-cast for contrast, must be subjected to high vacuum. For that matter, the electron microscope itself is evacuated. Specific examples of the kinds of problems on which this method can be tried might be how the conformation of the cortical cells is effected by setting the hair with chemical agents or the pitting and etching of scale fragments caused by different reagents.

As an example of the kind of pictures one can obtain, Fig. 10 shows a fragment of fibrillar material from a cortical cell at 10,000× magnification. In this micrograph, the fibrillar nature of the cortical cells is very evident. The larger fibrils at the right have been broken down far enough to see microfibrils. Figure 11 shows a fragment of a cuticle cell. Note the non-fibrillar nature of this as contrasted with the cortical cell. The proteinaceous material within the cell has been partially removed from this one as evidenced by the many pits appearing in the surface.

In the application of the various techniques which have been described to hair, it is always well to use both the light and electron microscope. The use of the ordinary light microscope can help one to better visualize what is being observed with the much higher magnification of the electron microscope.

Summary

The three techniques of replication, ultrathin sectioning and degradation represent the most useful techniques for examining hair at the present time. Their usefulness in any particular problem is limited only by the imagination, skill and care of the individual applying the techniques. Often a combination of two or all three techniques must be used. It is certain that the techniques will be modified and improved and that new techniques for examining hair in the electron microscope will continue to be developed as more people become interested in the application of the electron microscope to the solution of problems which may arise in the study of hair.

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