ELECTRON MICROSCOPY OF COLLAGEN FIBRILS DURING CORTISONE THERAPY OF ALOPECIA TOTALIS (TECHNICAL PROBLEMS)*

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Previous investigation has revealed that cortisone or corticotrophin tend to produce a remission in cases of alopecia totalis (1, 2, 3). Clinical observations on certain diseases of the mesenchymal tissue e.g. lupus erythematosus and rheumatoid arthritis, indicate that these diseases also tend to undergo remission following the use of cortisone or corticotrophin (4). This latter group of diseases has several findings in common, one being that the tissue generally affected is collagen (5). Hence, the authors used the electron microscope to determine:

(a) If the morphology of the collagen fibrils is altered in alopecia totalis, and

(b) If the collagen fibrils are altered following the administration of cortisone for the treatment of this condition.

PROCEDURE

Punch biopsy was done on the affected scalp and a similar biopsy was performed on the skin of the back for control observations. Another biopsy was performed on the scalp of normal subjects for further comparison.

Three patients with alopecia totalis were the subjects for study. Biopsies were obtained before therapy was begun and again at 6 and 12 week intervals after the initiation of cortisone treatment, at which time there was a regrowth of hair.

TECHNIC

Biopsy material from the three patients and one normal control was treated in the following manner (6, 7): Connective tissue was teased by gross dissection from the dermis into 25 ml. citrate-phosphate, pH 8, buffered distilled water. This tissue suspension was then subjected to sonic oscillation for 10 minutes; and a 4 hour digestion at 37° C. with 1% trypsin to remove amorphous material from the collagen fibrils. The digestive action was stopped and the material was fixed by the addition of 0.5 ml. 2% osmium tetroxide to the tissue suspension. After remaining 12 hours at 4° C. the suspension was centrifuged at 10,000 r.p.m. for 10 minutes. The sediment was triple-washed in pH 7.4 citrate-phosphate, distilled water and condensed to 5.0 ml. The more gross material was then settled by centrifuging at 2500 r.p.m. A micro-pipette drop was placed on a collodion-filmed stainless steel grid, allowed to dry and then washed 3 times with distilled water and redried. The grids were shadowed with chromium according to the method of Williams and Wyckoff (8). A RCA-EMT electron microscope with a magnification of × 6000 was used. The electron micrographs were then enlarged photographically × 3 to reach a final magnification of × 18, 000.

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The second biopsy from the normal scalp was used to control technical factors, i.e. some specimens were prepared in the usual way, others were prepared without sonic oscillation and in some trypsin digestion was omitted from the procedure. In all specimens the grids were prepared, shadowed and examined in a uniform manner.

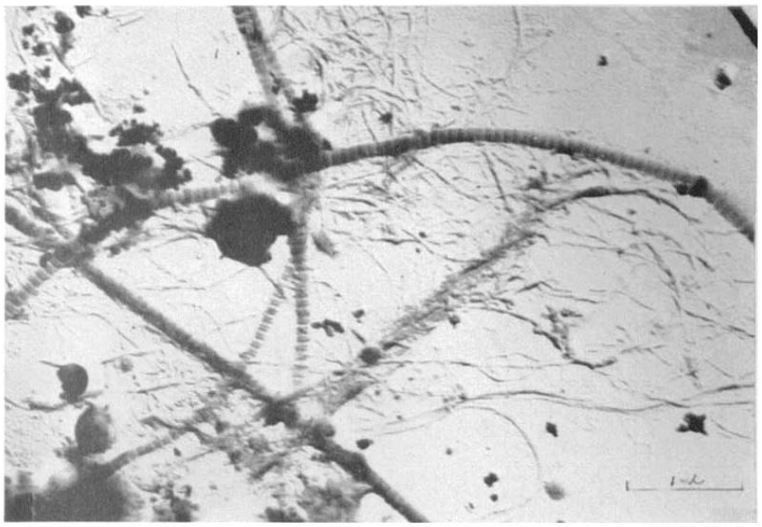

FIG. 1. Electronmicrograph of connective tissue from disease site prior to treatment

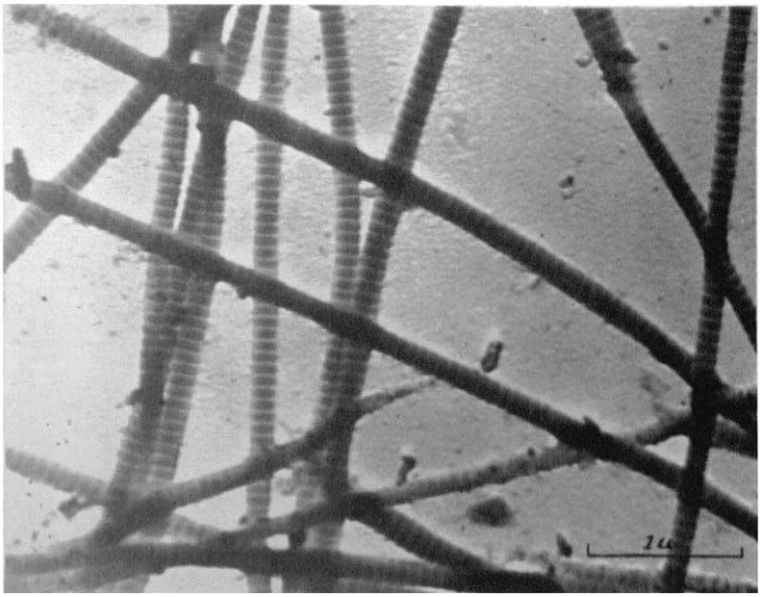

FIG. 2. Electronmicrograph of connective tissue from a control site on the back of same patient.

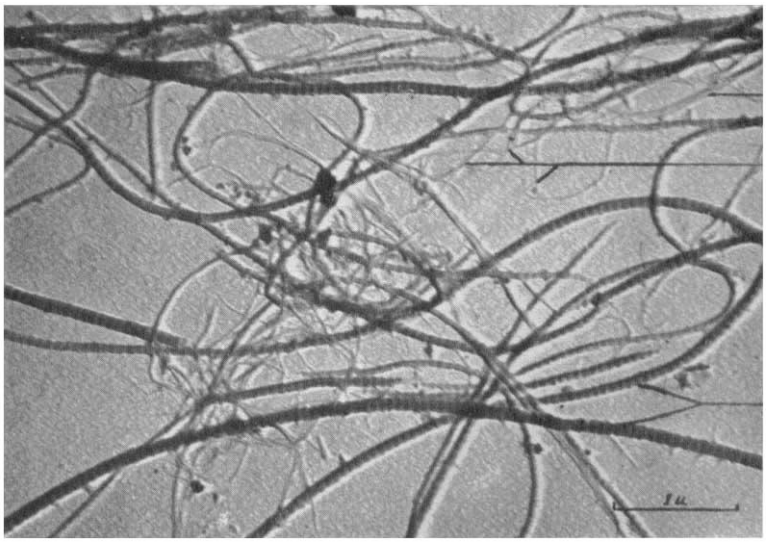

FIG. 3. Electronmicrograph of connective tissue showing collagenous filaments partly fragmented as well as normal collagen fibrils.

RESULTS

In the biopsy examinations the only suggestive evidence of alteration in the collagen structure in alopecia was the presence of abundant collagenous filaments in the disease-site biopsy as contrasted to the skin from a control site on the back of the same subject (Figs. 1 and 2). However, similar filamentous material was seen to some degree in the specimens from the scalp of a normal subject. We therefore questioned the suggestion that the filamentous material was characteristic of the disease process. In considering what significance could be attached to the presence of this material we decided to re-study the specimens prepared for the control of technic. This revealed the presence of the filamentous material (Fig. 3) only in those specimens where sonic oscillation and trypsin digestion were used together. From this we concluded that the filamentous material was not characteristic of the disease process. P. Vanamee (9) has noted a similar finding during the use of trypsin for this purpose.

SUMMARY

1. The electron microscope has been used to study the collagen tissue obtained from biopsy material in three cases of alopecia totalis before and after cortisone therapy.

2. These observations did not indicate any alteration in the physical structure of collagen fibrils in alopecia totalis before treatment or during the use of cortisone.

3. Attention is drawn to the misleading results which may be produced by the use of sonic oscillation and trypsin digestion in the preparation of skin biopsies for electron microscopic studies.

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