DIRECT VISUALIZATION OF A MUCOPROTEIN COMPONENT OF URINE

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PLATE 1

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Previous reports (1–4) have described a mucoprotein obtained from human urine by a procedure of precipitation with 3.4 per cent NaCl. It is characterized by a very high molecular weight (4) and by an extraordinarily high axial ratio (2, 4). These unusual features made it seem that direct visualization of the thread-like molecules with an electron microscope would be possible and that this might provide information as to the structure of the unit particle. It was felt that such information should be of considerable significance, since very mild procedures were used in isolating the material and since, in preparation for electron microscopy, it was dried from solution in distilled water.

EXPERIMENTAL

The mucoprotein studied was isolated (1, 2) from either male or female human urine by precipitation with 0.58 M NaCl at 4°. The precipitate which formed was washed with fresh NaCl solution and then dissolved in distilled H_2O. Traces of the original salt were removed by exhaustive dialysis. The distinctly viscous solution obtained was centrifuged at 3800 × *g* for 30 minutes to remove particulate contamination, and the concentration of the mucoprotein in the supernatant solution was determined spectrophotometrically (2). This original solution (4) was then diluted with freshly distilled H_2O to give final concentrations of 0.25, 0.025, and 0.0025 mg. per ml. Small drops of these solutions were placed on separate collodion-coated grids, all fluid in excess of a shallow drop was withdrawn, and the preparations were allowed to dry or were first placed in vapors of OsO_4 and then allowed to dry.

The dry preparations were shadowed with platinum-palladium at a 12.5° angle or 17° angle and thereafter examined. The majority of micrographs were taken at a magnification of 13,000 diameters.

Results

The preparations were consistent in showing long and slender filamentous units. When the original concentration was 0.25 mg. per ml., these

generally formed upon drying dense mats in which the individual filaments were just perceptible. Preparations from less concentrated solutions showed correspondingly fewer filaments per unit area. The concentration varied to some extent from one open area of the grid to another owing to the amount of original solution available over the area for drying, but this variation never exceeded the differences between the 10-fold concentrations. The definite correspondence between the number of filaments in dried preparations and the concentration of the solution made it evident that the filamentous component represented the mucoprotein. In most preparations the filamentous component was the only one apparent, but even in the few instances in which another was present it was not filamentous and was easily recognized as a contaminant.

Judged from their appearance in the dry preparation, the individual filaments are non-rigid strands with an average diameter of ~100 A. In these features they are uniform within a single preparation as well as from one preparation to another. In length, however, the variation is great. Some filaments measure as little as 2500 A; others as much as 40,000 A or 4 μ. The majority are greater than 15,000 A in length, and most of these fall in the range between 20,000 and 30,000 A. It is probable that the preparation procedures operated to fragment many filaments that were longer in the original material. However, since the procedures used were extraordinarily mild, it seems unlikely that they were totally responsible for the variation. Regardless of its origin, this degree of variation was a constant feature of the preparations and appeared to bear no relation to whether OsO_4 was used as a fixative or not. These dimensions described a highly asymmetric body with an average axial ratio of approximately 250. It should be noted that preparations from male and female urine were indistinguishable by electron microscopy.

It was characteristic for the filaments to appear beaded in preparations shadowed lightly with platinum-palladium. When this structure was evident in the filaments (and the clarity of this nodose structure varied considerably and was ill defined in some places), it appeared to be quite regular with high points spaced at ~110 A. This suggests that the filaments are linear arrangements of spheroidal particles and that these latter may represent the fundamental, repeating unit of the mucoprotein.

It is interesting to note that in water solutions the filaments of mucoprotein showed no tendency toward lateral association. Indeed they appeared to resist such association under these conditions for it was rare, even in the dried preparations from 0.025 mg. per ml. (Fig. 1), to find two or more filaments lying side by side. When, however, the mucoprotein was dissolved in 0.85 per cent NaCl, it had a tendency over long periods of storage at 4° to precipitate (1, 2). When such salt solutions were

placed dropwise on electron microscope grids and allowed to dry, the mucoprotein filaments were found to be laterally associated in bundles and the whole preparation resembled a fibrin clot, similarly examined (5). Presumably the increase in salt concentration accompanying the desiccation of the material accelerated this lateral aggregation of the filaments.

DISCUSSION

It is evident from the electron micrographs that the filamentous component of these preparations of mucoprotein has an asymmetry adequate to account for the high viscosity of the preparations. To what extent this asymmetry varies under normal conditions is in doubt, but sedimentation data (4) would suggest that axial ratios are more uniform for the material in solution than after drying. Presumably the forces of surface tension operating in desiccation would serve to fragment the filaments.

The width of the filaments seems remarkably uniform, and in this regard the observations reported here conflict to some extent with those reported earlier on ovomucoid by Sharp, Lanni, and Beard (6). The filaments resemble in their diameters and in their electron microscope image the appearance of F-actin (7) as well as a number of filamentous units found within cells, such as myofilaments (8) and the tonofibrils of epidermal cells (9).

Calculations based on viscosity, sedimentation, and diffusion indicate that the dimensions of these mucoprotein filaments are 5600 A in length and 42 A in width (4). Between these dimensions and those obtained from electron microscope images there is an obvious discrepancy. From the latter evidence the average dimensions are 25,000 A in length and 100 A in width. A number of suggestions might be made to account for these differences. First, for physicochemical studies the phosphate buffer-soluble fraction of lyophilized mucoprotein (2, 4) was used, whereas for electron microscopy the mucoprotein had not been lyophilized and was used in a state in which it was completely soluble in phosphate buffer (4). Secondly, in making calculations of the dimensions from physicochemical data, a uniform rod-like model was utilized. The electron microscope indicates, to the contrary, that the filaments are nodose as though made up of particulate elements. Obviously, a beaded filament of the same volume would have greater dimensions than a rod. Finally, the electron microscope images define the filament as a flexible structure, with little evidence of the rigidity which is assumed in calculations from viscosity, sedimentation, and diffusion data.

There is nothing in these images to indicate that the filaments are in any sense rigid or responsive one to the other. On the contrary, they twist and turn as though completely flexible. Parallel or lateral associa-

tions of the filaments have not been encountered, and instead the orientation is entirely random. It would seem then that in water solution the mucoprotein filaments show no tendency to lateral aggregation. Only when the concentration of NaCl is increased to about 0.14 M do the filaments come together to form a precipitate.

Although the filaments appear to behave as units in these preparations and also in response to electrophoretic fields and changes in H^+ ion and salt concentrations, the electron microscope image indicates that they are nodose and apparently constructed of spheroidal units. These units have approximately the same length as the filament width. The integrity which the filaments maintain in the face of the forces of drying would suggest that the bonding between the particles is strong. Presumably when subjected to conditions which reduce the viscosity of the solution, such as 70° for 30 minutes (2, 4), the filaments fragment. Thus far electron microscope studies of such material have not been attempted. The observation that these filaments apparently consist of spheroidal particles adds yet another item to the list of highly asymmetric "molecules" which appear to be constructed in this manner (10). Electron microscope evidence defines fibrinogen (11) and the first polymeric forms of fibrin as strings of particles (5, 11). Apparently F-actin (7) filaments are similarly constructed, as are also keratin filaments (12) and insulin (13). Sharp, Lanni, and Beard stated that filaments seen in semipurified preparations of the egg white inhibitor of influenza virus hemagglutination appeared to be linear aggregates of spheroidal particles (6).

From the information available it would appear that this component of urine is derived from the walls of the bladder or urethra. That it is not added to the urine solely during its passage through the urethra is indicated by its presence in urine catheterized from the bladder. Presumably the mucous cells to be found in the transitional epithelium around the base of the bladder represent the source in this case. Cells of this same type are not present in the ureter at any level, and it seems very unlikely that any filament of these dimensions could find its way through the nephron even if present in the plasma. Likewise the secretory cells of the genital tracts and glands cannot be implicated, because the same material is found in female as in male urine. The most logical source would appear, therefore, to be the mucous cells of the bladder, with possible additions from the glands of Littré found in the walls of the urethra. There is ample reason to believe that the site could be identified by electron microscopy of thin sections taken through the mucous cells of the bladder and urethra, provided its intracellular form bears some resemblance to the extracellular appearances. Such investigations might reveal also whether mucous secretions are uniformly characterized by unit filaments of this long, slender nature.

One of the more interesting properties of this material, as indicated in earlier publications (1–4), is its capacity to combine temporarily with and be permanently altered by influenza, mumps, and Newcastle disease viruses. There can be little doubt that the filament pictured in these micrographs is involved in this reaction. Heretofore, electrophoretic (3) and ultracentrifugal (4) studies have defined the purity of the preparations and related activity to concentration. The electron microscope observations do essentially the same thing and add new evidence on the morphology of the particles.

SUMMARY

Samples of urinary mucoprotein have been examined with the electron microscope and have been found to be made up of filaments having a width of ~100 A and an average length of ~25,000 A. They appear to be nonrigid. The filaments show a nodose structure, with high points spaced at ~110 A, indicating that they may be composed of spheroidal units.

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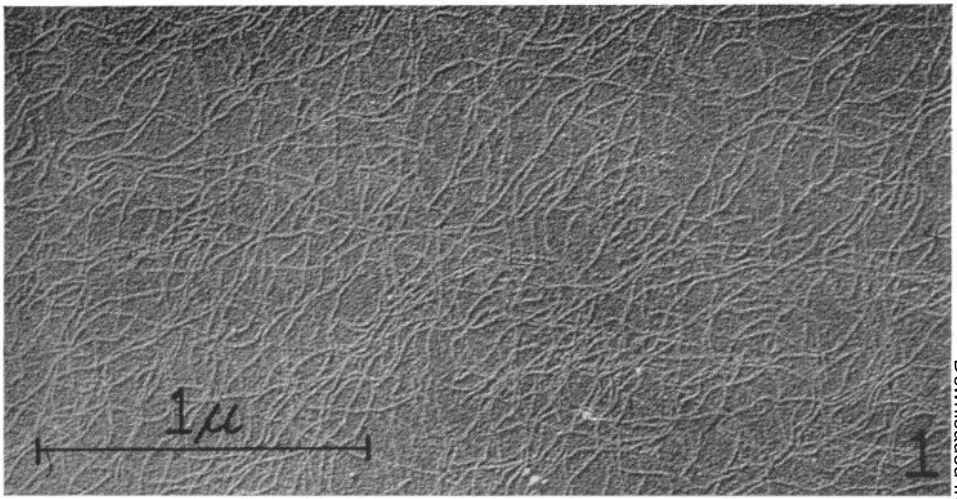
EXPLANATION OF PLATE 1

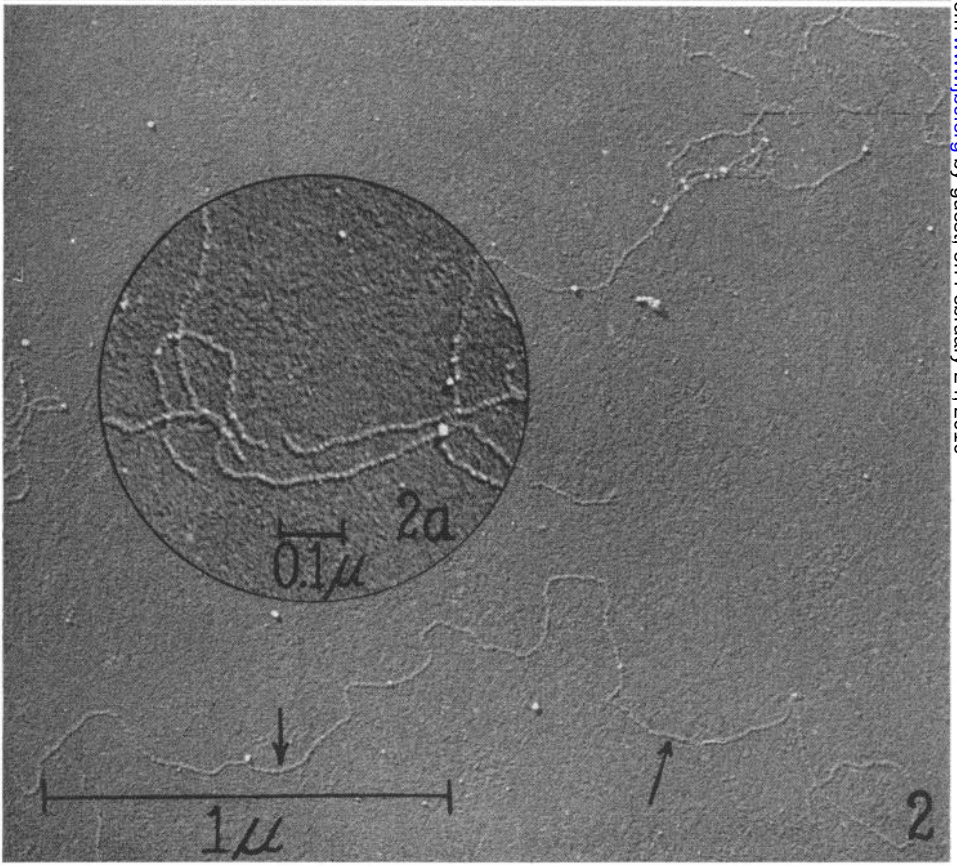
FIG. 1. Electron micrograph of a preparation of mucoprotein (from male urine) dried from a water solution containing 0.025 mg. per ml. The solution was placed in a shallow drop on a collodion-coated grid and then allowed to dry. Osmium fixation was *not* employed. When dry, the surface of the preparation was shadowed with platinum-palladium at an angle of 12.5°. Magnification, × 43,500.

FIG. 2. Micrograph of mucoprotein (from female urine) dried from a solution containing 0.0025 mg. per ml. A shallow drop of the solution was placed in vapors of OsO_4 to "fix" the mucoprotein. After 10 minutes it was removed from the container of OsO_4 and allowed to dry. It was shadowed at an angle of 17°. It is evident that the 10-fold dilution of the solution is reflected in the relative sparseness of the filaments. What appears to be a single filamentous unit stretches across the

bottom of the micrograph (arrows). It is 4 μ long. It may be noted that the filaments in Fig. 2 do not appear larger than those in Fig. 1 even though the magnification is greater. This is explained by the fact that the filaments in Fig. 1 were shadowed at the smaller angle of 12.5°. Magnification, × 53,625.

FIG. 2, *a*. Micrograph of mucoprotein (prepared as for Fig. 2) at higher magnification to show the nodose character of the filaments. The average width of the filaments is 100 A, and the height, as determined from shadow length and angle, is approximately 30 A. Assuming a circular cross-section, it would appear that in spite of fixation some flattening of the filament results from the forces of drying and adsorption. Magnification, × 78,000.





(Porter and Tamm: Mucoprotein from urine)