Independence of Urinary Acid Mucopolysaccharides and Tamm-Horsfall Urinary Mucoprotein.* (28751)

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Di Ferrante and his coworkers(1) were the first to use the quaternary ammonium compound, cetyltrimethylammonium bromide (CTAB), for precipitation of urinary acid mucopolysaccharides. Even after extensive purification their analytical results(2,3), as well as those of others(4-6), indicated the presence of proteinaceous material. The only protein identified in the CTAB precipitate (2,4,6) is the Tamm-Horsfall urinary mucoprotein(7). A possible role of a protein-acid mucopolysaccharide complex in urolithiasis makes it of interest to ascertain whether some of the Tamm-Horsfall mucoprotein is in fact attached to urinary acid mucopolysaccharides or if it is merely co-precipitated.

Materials and methods. Four experiments were performed: (a) The first experiment was intended to determine whether a CTAB precipitate could be isolated that was free of Tamm-Horsfall mucoprotein. A sample from normal urine was purified according to the procedure of Di Ferrante and Rich(8), up to the point of treatment with enzymes. The product was further treated with 0.58 M NaCl at pH 4.5 and centrifuged, which should remove Tamm-Horsfall mucoprotein (7). The dialyzed and lyophilized supernatant was examined immunoelectrophoretically(9) for Tamm-Horsfall mucoprotein.

(b) Tamm-Horsfall mucoprotein, prepared by NaCl precipitation(7), contains no hexuronic acid. Ten mg of chondroitin sulfate was added to 4.15 mg of such a preparation, in 10 ml water. The chondroitin sulfate (from beef trachea) gave a single Tiselius electrophoretic peak (plus an immobile boundary artifact) at pH 8.6 and 4.5; analytical data on this same preparation have been published(4). After 30 minutes at room temperature, the mixture was made 0.58 M in NaCl. The resulting precipitate was washed 3 times with 0.58 M NaCl and examined for hexuronic acid(10). As little as 10 μg would have been detected.

(c) Ten mg of CTAB precipitate of human urine, prepared according to Maxfield (6), was dissolved in 5 ml physiologic saline and 2 ml of rabbit antiserum added. After 30 minutes the antigen-antibody precipitate was centrifuged, washed, and examined for hexuronic acid as in (b).

(d) Whole fresh urine, 10 ml, had 2 ml Tamm-Horsfall antiserum added. The antigen-antibody precipitate was similarly examined.

Antisera to products of the Tamm-Horsfall or Maxfield procedures were prepared by subcutaneous injection into rabbits of 3 mg antigen in 1 ml water plus 1 ml Freund's complete adjuvant (Difco) for 4 successive days. The injection series was repeated 2 further times at 2-week intervals and the animals bled 2 weeks after the last injection. Three rabbits were used for each antigen. The antisera gave a single strong precipitin line with the original antigens, either on Ouchterlony plates(11) or microimmunoelectrophoresis(9) in agar gel. A "line of identity" was obtained when materials prepared by either method were diffused against either antiserum in adjacent peripheral Ouchterlony wells. The antigens did not react with a rabbit anti-human serum which gave 14 precipitin lines immunoelectrophoretically with human serum. Only the Tamm-Horsfall precipitin line appeared on similar examination, using antisera to the total nondialyzable urinary solids which gave 4 to 12 precipitin lines with their own antigens.

Results and discussion. The product of the first experiment gave a strong precipitin line with antiserum to Tamm-Horsfall mucoprotein. It gave no reaction with anti-human serum; urinary total nondialyzable solids

* Supported by Deutscher Akademischer Austauschdienst and by USPHS, NIH grant.

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antiserum disclosed the presence only of Tamm-Horsfall mucoprotein. Thus, the purification had not completely removed Tamm-Horsfall mucoprotein and no other component was detectable. The purified material contained 5.9% N (Dumas) and 18% hexuronic acid; the theoretical values for pure chondroitin sulfate are 2.7% and 37.3% respectively.

No hexuronic acid was found in experiments *b* to *d*.

In the first experiment, the persistent presence of Tamm-Horsfall mucoprotein suggested that it is complexed with the acid mucopolysaccharides, and analogous to chondromucoprotein. The absence of hexuronic acid from the materials examined in experiments *b* to *d* demonstrates that Tamm-Horsfall mucoprotein in urinary CTAB precipitates, is actually co-precipitated, none being bound to urinary acid mucopolysaccharides.

Summary. Tamm-Horsfall mucoprotein, found with urinary acid mucopolysaccharides prepared by precipitation with cethyltrimethylammonium bromide, is present adventitiously as a coprecipitated impurity rather than as bound moiety. The nature of any actual proteinaceous moiety is unknown. None is immunologically detectable with antisera to proteins of human serum or urine.

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Received September 3, 1963. P.S.E.B.M., 1963, v114.

Growth Requirements of *Saccharomyces cerevisiae* as a Result of Incubation at Increased Temperature.* (28752)

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An earlier report from this laboratory(1) showed that *Saccharomyces cerevisiae* (Fleischmann strain 139) grew poorly or not at all in a chemically defined medium at 38°C while growth was excellent in the same medium at 30°C. Addition of yeast extract to the defined medium permitted growth at the increased temperature of incubation, as reported also by Sherman(2). Other workers (3-6) have associated specific substances as required growth factors at increased incubation temperatures, and further efforts on our part demonstrated that pantothenic acid could largely replace the effect of yeast extract. Pantoic acid also was found to be effective but only at concentrations 100 to 10,000 times as great as pantothenate.

The present paper considers the growth requirements of a mutant isolated from the original Fleischmann strain of *S. cerevisiae*.

Materials and methods. The experimental procedure was described previously(1). Inoculum per tube was 0.05 ml of a washed cell suspension having a turbidity of 30 in a Klett-Summerson photoelectric colorimeter (660 mμ) using water as a blank.

* These studies were aided by a contract between Office of Naval Research, Department of the Navy, and University of Cincinnati and the National Science Foundation.