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A Carbohydrate Isolated from Monilia Psilosis.

D. H. COOK, H. D. KESTEN AND J. W. JOBLING.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City, and the School of Tropical Medicine of the University of Porto Rico, under the auspices of Columbia University.

In the light of the recent work on specific carbohydrates of various organisms, it seemed advisable to test the nature of one of the *Monilia* group of fungi in this respect. *Monilia psilosis,* Ashford, was chosen because of its possible relation to tropical sprue. Kurotchkin and Chu,[1] using a *Monilia tropicalis,* isolated from a case of bronchomoniliasis, obtained complement fixation and weak precipitin tests with an alkaline extract of the organism and with the alcoholic precipitate from this extract. No further attempts at purification of this material were reported.

The method of extraction, as first used, was that of Salkowski as given by Mueller and Tomcsik[2] in their work on yeast gum. Six day cultures of *Monilia psilosis* grown on honey broth were centrifuged, washed with water followed by alcohol, and extracted over-night twice with ether. The organisms, when treated by the method of Mueller and Tomcsik, gave an active material as indicated by the precipitin reaction. The method was later modified in that the monilia bodies were given (after alcohol washing and ether extraction) a preliminary treatment with Fehling's solution, 100 cc. for every 10 gm. of the dry material. The violet-colored material was centrifuged off and washed 3 times with distilled water, thus removing considerable protein dissolved by the alkali. The colored residue was then decolorized with N HCl and centrifuged or fil-

[1] Kurotchkin, T. J., and Chu, C. K.; *Nat. Med. J. China,* 1929, xv, 403.

[2] Mueller, J. H., and Tomcsik, J., *J. Exp. Med.,* 1924, xl, 343.

tered. The clear liquid contained the active material. This liquid was treated with Fehling's solution, which caused the separation of a blue, flocculent precipitate, the active material. This, after two washings with distilled water by centrifugation, was dissolved in 10 cc. of N HCl for each 10 gm. of the original monilia. If the solution was not entirely clear, 95% alcohol was added to the point of first increased opacity, generally about one volume. Prolonged centrifugation threw down impurities, leaving a water-clear solution. From here on the technique was that used by Mueller and Tomcsik, consisting of repeated precipitation with 3 or 4 volumes of alcohol from dilute acid solution, followed by washing and drying with ether.

The final product is a white, friable material, which gelatinizes and slowly dissolves in water. It gives a Molisch test in a dilution of 1:1,000,000. The best sample contains 0.58% N,* and though it gives a faint bluish-red biuret in 2% solution on standing several minutes, the Hopkins-Cole, xanthoproteic, Robert's, trichloracetic, Millon, and ninhydrin tests are all negative. Where possible, the tests were carried out upon the solid material to increase sensitivity. It is precipitated by basic lead acetate or glacial acetic acid, but not by neutral lead acetate. From these results it would appear that the active material is non-protein in nature, though the presence of the nitrogen makes it impossible to state this with finality. Sufficient material has not been available to do a Van Slyke amino-nitrogen determination.

The carbohydrate gives no reduction on boiling with Fehling's solution, but after 15 minutes' hydrolysis with N HCl at 100°, reducing sugars are present. Glucosazone was prepared from the hydrolysate and identified by its crystalline form. Glycogen is absent by the iodine test. The naphtholresorcinol test gives a reddish-brown ether solution showing two absorption bands, one in the red, the other in the green, thus indicating the possibility of a uronic acid linkage. Lack of material has hindered more complete investigation of the chemical nature of the carbohydrate. Calculated on the dry weight of monilia, the yield of purified carbohydrate is about 1%.

Using an antiserum prepared in rabbits by the injection of a suspension of killed *Monilia psilosis*, and having an agglutination titer of 1:400, precipitin tests were done on various preparations of the carbohydrate. Precipitin rings were regularly obtained in dilution of 1:100,000 in ½ hour and often within five minutes. Occasionally a ring was formed in one hour at a dilution of 1:1,000,000. Nor-

* Dumas micro-nitrogen determinations were kindly done in the laboratory of Professor J. B. Niederl, Washington Square College, New York University.

mal rabbit serum and anti-sera prepared against a strain of *Willia* and against Fleischmann's yeast were negative at 1:100 dilution of the polysaccharide. The activity of a 1:100,000 solution was apparently undiminished after heating 30 minutes at 100° C.

In view of the specificity of most of the other polysaccharides isolated by various workers it is of interest to note that precipitin rings were obtained with *Monilia psilosis* polysaccharide using antisera† prepared against similar yeast-like organisms, but either at a lower dilution or less rapidly, in the majority of cases. (These included *Monilia albicans, Monilia parapsilosis,* Ashford, *M. krusei,* Castellani, a non-pathogenic monilia, and two strains of monilia isolated from erosio interdigitalis.)‡ This raises a possible question as to the individuality of the species so far tested and is further confirmation of the findings of Hopkins and Benham,[3] who observed cross-agglutinations among these same organisms, although certain distinctions could be obtained by agglutinin absorption. Work is in progress to obtain the carbohydrates from related species with the object of studying cross-precipitin reactions and precipitin absorption.

With the isolation of additional active material it is hoped to amplify both the chemical and immunological studies including skin and serum reactions of individuals harboring monilia.

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The Dietary Production of Dystrophy of the Voluntary Muscles.*

MARIANNE GOETTSCH. (Introduced by A. M. Pappenheimer.)

From the Laboratory of Biological Chemistry, College of Physicians and Surgeons, Columbia University of New York.

Vitamin E has been shown by Evans, Bishop, and their coworkers[1, 2, 3] to be necessary for normal reproduction in the rat, and by

† Kindly furnished by Miss R. W. Benham, Department of Dermatology, College of Physicians and Surgeons, Columbia University.

‡ It is interesting that the monilia polysaccharide precipitated with a sample of Type II pneumococcus antiserum furnished by Dr. M. Heidelberger.

[3] Hopkins, J. G., and Benham, Rhoda W., *N. Y. State J. Med.*, 1929, xxix, 793.

* This work has been conducted with the aid of the departmental Research Fund of the Chemical Foundation.

[1] Evans, H. M., and Bishop, K. S., *J. Met. Research*, 1923, iii, 201, 223.

[2] Evans, H. M., and Burr, G. O., *Memoirs of U. of Calif.*, Berkeley, 1927, viii.

[3] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, lxxvi, 273.