were always sterile. It is interesting to point out that in this study no appreciable difference in invasiveness was noticed in any of the 3 types.

The result of the present study seems to indicate that in Chinese hamsters, at least, the organisms classified as the *gravis* type showed neither greater pathogenicity nor enhanced invasiveness. Whatever difference that was obtained must be attributed to the difference in the individual strain rather than to the difference in types.

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## Specific Carbohydrate from *Asterococcus mycoides* for Serologic Tests of Bovine Pleuropneumonia.

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It is known that a substance active in the specific precipitative reaction is present in the serum of infected animals and can also be extracted from the affected lungs in cases of bovine pleuropneumonia. These observations suggest the presence of a haptenic carbohydrate. An attempt, therefore, was made in the present experiment to isolate a specific carbohydrate from pure cultures of *Asterococcus mycoides,* the causal agent of bovine pleuropneumonia, and to determine its serologic activity with immune rabbit's serum.

The strain of the organism used here was isolated from the pleural exudate of a fatal case of pleuropneumonia in a cow. Forty flasks, each containing 200 cc. of liver-digest broth enriched by addition of 5% unheated horse serum, were inoculated with the organism and incubated for 5 days at 37°C. The growth was centrifuged for 2 hours at 3,000 r.p.m. and the sediment was collected, washed in saline, centrifuged again and finally resuspended in distilled water. For the sediment obtained from one liter of culture 20 cc. of water was employed. This suspension was left in the icechest overnight. At the end of this period, 0.5% of potassium hydroxide was added following which the suspension became transparent and markedly slimy. This was centrifuged and to the clear fluid glacial acetic acid was added. A floccular precipitate, designated here as nucleoprotein, developed. The precipitate was collected, dried in the incubator and preserved for serologic tests. The remaining clear fluid

was passed through a Seitz filter and further precipitation accomplished by addition of 5 volumes of 95% ethyl alcohol. The mixture was left in the icechest for 18 hours. During this time a small amount of white precipitate was formed. This was collected, dried in the incubator and used in the serologic tests. Because of the small amount of the substance, the yield of which was 10 mg. per liter of culture, no further purification through reprecipitation with alcohol has been attempted.

In chemical tests the substance gave negative biuret and positive Molisch reactions.

Tests for precipitin and complement-fixation were carried out with rabbit-antiserum prepared by immunizing with washed cultures of *Asterococcus mycoides*. The serum in 1:80 dilution agglutinated the organism. The interfacial test was used to detect precipitin and Kolmer's technic was employed in the complement-fixation test. Undiluted serum was used in the precipitative test, and a 1:5 dilution in the complement-fixation test.

The formation of a distinct ring occurred when the substance was diluted to 1:25,600, while complete inhibition of hemolysis occurred in 1:102,400 dilution of the substance. Nucleoprotein from *Asterococcus mycoides* was also tested serologically. It was found that no precipitation occurred when nucleoprotein was used in a concentration of 1:200, but complete inhibition of hemolysis was observed in dilution 1:6,400. A washed suspension of *Asterococcus mycoides* was also used in the complement-fixation test and positive reactions occurred only when not less than 0.5 cc. of the suspension equivalent in density to the original culture was used.

The data suggest that the substance prepared from pure cultures of *Asterococcus mycoides* is a specific carbohydrate having characteristics similar to those derived from other organisms. The high specific sensitivity of the substance indicates the possibility of its use for diagnostic purposes especially for the detection of chronic cases of bovine pleuropneumonia.