Serological studies of moth proteins with special reference to specific immune bodies and their phylogenetic significance
by Saxon Martin

A THESIS Submitted to the Graduate Committee In partial fulfillment of the requirements for the Degree of Master of Science in Botany and Bacteriology at Montana State College
Montana State University

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SEROLOGICAL STUDIES OF MOTH PROTEINS WITH SPECIAL REFERENCE TO SPECIFIC IMMUNE BODIES AND THEIR PHYLOGENETIC SIGNIFICANCE

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INTRODUCTION

The significance attached to immunological reactions and especially to the so-called specific immune body in immunological reactions of various bacteria has been presented by Krumweide and Noble (12), Heidelberger and Avery (8), Gunnison and Shoenholz (6), Hitchcock (11), Sugg and Neill (20), Lancefield (16), and others. The presence of these bodies in the higher forms has been demonstrated by Landsteiner (15) in his work on horse saliva.

A specific immune body has been isolated from collections of Feltia ducens Wlk. belonging to the family Phalaenidae. The writer has demonstrated that the precipitin reaction can be used to determine a phylogenetic relationship between members of this group.

There has been little work done on the specific immune bodies occurring in members of various phyla of the animal kingdom and no available literature as regards such work on insects.

Krumwiede and Noble (12) described a rapid method for the production of precipitin antigen from bacteria. Heidelberger and Avery (8) described a method used in obtaining the soluble specific substance from Pneumococcus in which they isolated the soluble specific substance by the concentration of broth cultures, precipitation with alcohol, repeated

re-solution and precipitation. This was followed by a careful series of fractional precipitations with alcohol or acetone after acidification of the solution with acetic acid and repeated fractional precipitation with ammonium sulphate and dialysis of the aqueous solution of the active fractions. Their material contained 1.2 per cent of nitrogen and yielded 79 per cent of reducing sugars on hydrolysis. The material gave a specific reaction in a dilution as high as 1:5,000,000.

Landsteiner and Levene (14) obtained an active substance from dried horse kidneys which inhibited hemolysis in high dilutions. Balls (2) found a considerable degree of specificity in identifying certain yeasts with the precipitin test. He suggested that a grouping of nearly related species could be made by this method. The precipitin reaction was found to be highly specific for two types of Bacillus botulinus van Ermengem and for the sub-groups within those types by Gunnison and Schoenholz (6).

Hektoen (10) worked with the precipitin reaction on body constituents and found that serum proteins, hemoglobin, and specific precipitinogen in serum were found to be specific for the species. Landsteiner and Levene (14) working on alcoholic extracts from horse kidneys obtained a water soluble substance which they purified with pyridine, chloroform, and alcohol. The final residue was hydrolyzed with sulphuric acid and the aqueous solution of the product of hydrolysis was titrated with Fehling's solution giving a reduction corresponding to 28 mg. of glucose. Hitchcock (11) in his work on the separation of a serological

group described a method in which he used sodium hydroxide to extract the antigenic substance from streptococci. Ando (1) used a modification of Heidelberger's and Avery's method in working with Streptococcus viridans Schotmuller, Bacillus dysenteriae Shiga, and Bacillus mallei Hoefler and Schutz and suggested its use in the diagnosis of glanders. Enders (5) found a type-specific substance in the autolytic products of Type I pneumococci distinct from the specific carbohydrates. He distinguished it from the carbohydrates by the fact that it was not stable when boiled in weakly alkaline solution. Tillet, Goebel, and Avery (21) worked on the chemical and immunological properties of a species-specific carbohydrate of pneumococci. They suspended the organisms in 50 cc. of saline and broke them down by repeatedly freezing and thawing them. The final solution after treatment with acetic acid and sodium hydroxide contained fraction C which was found to be non-specific.

Sugg and Neill (20) described a method for the extraction of antigens in their studies on the relationships among the pneumococci in which the antigen was prepared by boiling cells in .05 normal HCl and adding acetic acid to precipitate the proteins. The active substance was then precipitated with alcohol.

Landsteiner (15), working on horse saliva, obtained an active substance by treatment with acid and acetone and fractionation with alcohol, which gave a weak biuret reaction and on hydrolysis yielded 48.5% reducing sugar.

Zozaya and Wood (24) found that the polysaccharide and the "nucleoprotein" obtained from different types of meningococci, gonococci, and Micrococcus catarrhalis Pfeiffer, had similar immunological properties and were not type-specific. They also found that the carbohydrate-precipitable substance in immune sera appeared in the fourth month or more in the immunization of animals.

Zozaya (22) showed that polysaccharides can be rendered antigenic by haptogenic adsorption upon a colloid carrier. With the polysaccharide of Type III pneumococci he was unable with his method of immunization of rabbits in six weeks to produce any detectable protective antibodies, but was able to produce anticarbohydrate antibodies. All of the bacterial carbohydrates were non-antigenic when used alone. Zozaya (23) also found that the synthetic polysaccharide produced by Leuconostoc mesenterioides (Cienkowski) Van Tieghem from saccharose, reacted immunologically with antisera from pneumococci, some of the Salmonella and some of the types of Streptococcus viridans Bargen. He says further, "this immunological relationship is independent of the specific antipolysaccharide antibodies of these sera, suggesting the existence of a distinct antibody produced by an active group of the specific bacteria polysaccharide, which is similar or identical to the active group of the dextran polysaccharide." "These findings warrant the generalization that antigens need not be of a complex protein nature, but that some substance of a colloid nature is responsible for antigenicity. The colloid may in certain cases be a protein, but any colloid which can adsorb the specific